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No. 2.—THE SIGNIFICANCE OF (*a*) ACID-FAST BACILLI; (*b*) *BACILLUS COLI*, IN MILK.*

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(a) The Significance of Acid-fast Bacilli in Milk

Amongst the numerous bacterial species encountered in milk, the group known as "acid-fast bacilli" are doubtless the most important, particularly to veterinarians engaged in public health duties.

The significance of the group from a public health point of view rests mainly on the frequency with which one of its members, the tubercle bacillus, is found in milk, together with its undoubted responsibility for a considerable percentage of tuberculosis in man. The remaining members of the group, in so far as they are found in milk, have no pathogenic significance. Their importance, however, rests on the fact that certain of them present such striking resemblances to tubercle bacilli, that they may, should they occur in milk, give rise to uncertainty and confusion in diagnosis.

As the success of any policy for eliminating tuberculous milk from the general supply depends upon the ease, rapidity, and certainty with which we are able to recognise the presence of tubercle bacilli, it follows that it is of the utmost importance to determine the significance of such other acid-fast bacilli as may be found in milk, with a view to ascertaining how far the results of routine examinations may be vitiated by their presence.

Before proceeding to a detailed consideration of this question, it is important that we should have an understanding of what is meant by the term acid-fast, and to what organisms it is applied.

An organism is said to be acid-fast if, when it has been stained with such an intense stain as carbol fuchsin, it cannot be decolorised by the application of strong acids. The method in routine use for determining the presence of this property is the staining method of Ziehl Neelsen, with which you are all familiar.

Whilst this acid-fast property was first associated with the tubercle bacillus, the widespread use of this staining method has shown that acid-fastness is also shared by a number of other organisms in whose structure a relatively large amount of lipoid or waxy material is present. Such organisms have consequently been grouped together into what is universally recognised as the acid-fast group.

This does not mean that acid resistance is a property exclusive to this group, and is absent in other bacteria. As will be pointed out later, acid-fastness can be found to a degree, under certain circumstances, in other organisms. It is admitted, however, that the degree of acid-fastness of non-members of this group is but a small one, but, at the same time, it may be sufficiently prominent, if due care in decolorisation is not exercised, to mislead one in diagnosis.

With reference to members of the group itself, it is well-known that the tubercle bacillus in particular is also resistant to the decolorising action of alcohol, a property which was thought to be absent in the case of the non-pathogenic acid-fast species. This finding led to alcohol being adopted as the criterion for distinguishing between the pathogenic and saprophytic acid-fast bacilli, and, by combining the processes of acid and alcohol decolorisation it was thought that we had a simple and reliable method of distinguishing between the pathogenic acid-fast bacilli and bacteria in general.

Further work and experience in searching for tubercle bacilli have shaken to some degree the confidence which we were accustomed to place in this test, as it has been shewn that certain saprophytes exhibit a similar resistance to acid and alcohol.

In connection with Ziehl-Neelsen staining, I would like to remind you that although there is considerable uniformity in the use of stains for the test, there is probably considerable variation in the process of decolorisation. Some use sulphuric acid or hydrochloric acid in varying strengths, either alone, or in conjunction with alcohol or methylated spirits, whereas some may even use other acids, such as nitric acid. If such is the case, it is not unreasonable to expect slight variations in the results, and an organism which may be slightly acid-fast to one method may take the counterstain in the other.

Just as there may be slight differences of fastness according to the strength of acid or alcohol

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used, it is probably equally true to say that an organism may display slight differences of fastness as a result of some variation in its own structure, or on account of the medium or tissue in which it has been growing. Space will not permit me to go further into this subject other than to mention that this has been noted in routine work, and in the cultural study of acid-fast bacilli in general (Arkwright, 1930, Miller, 1932).

From this it will be appreciated that so long as such variations may happen, infrequent and comparatively insignificant though they may be, one cannot depend on staining reactions alone to distinguish between one organism and another.

Despite these limitations, however, the use of acid and alcohol as decolorising agents in the Ziehl-Neelsen method still provides us with the most simple and efficacious method of demonstrating microscopically the presence of pathogenic species.

Having discussed the significance of the term acid-fast, I now wish to draw attention to the organisms in which this property is present in an unusual degree, namely the acid-fast group. They constitute the Genus *Mycobacterium* Lehmann and Neumann, 1896, and include numerous members pathogenic to man and animals, and also certain saprophytic species.

The following are the chief pathogenic members:

Mycobacterium tuberculosis (human).

This is the most frequent cause of tuberculosis in man. It is rarely encountered in naturally-acquired tuberculosis in cattle, and even under experimental conditions, is incapable of setting up a progressive condition. Its presence in milk, therefore, indicates contamination from a human source.

Mycobacterium tuberculosis (bovine).

This organism is the cause of all forms of progressive tuberculosis in cattle, and, through the agency of milk, can be held responsible for a considerable proportion of tuberculosis in other animals and man. The bovine organism plays a very prominent part in human tuberculosis, and is responsible for considerable mortality and invalidism amongst children.

Under what conditions are bovine tubercle bacilli found in milk? In the majority of cases their presence is associated with clinical tuberculous mastitis. They may, however, be encountered in the milk of cows in which there are no detectable lesions in the udder. This may occur in what is known as the pre-clinical stage of tuberculous mastitis or, as appears to be established, the bacilli may pass from the system into the milk through the healthy udder. That this is possible has been noted by Weber and Titze (1907), A. S. Griffith (1911), Gofton (1921), and others. E. A. Watson (1931) states: "It is often asserted that, as a rule, the milk of tuberculous cows which do not have active tuberculosis of the udder is free from tubercle bacilli and harmless, and that milk is not dangerous until the udder itself becomes diseased. That itself is a fallacy and a dangerous one. . . . It has been proved, over and over again, that tubercle bacilli may be excreted with the milk from an udder in which neither manifest tuberculosis nor microscopical evidence of tuberculous lesions is present. . . . We have determined that cattle may harbour and excrete virulent tubercle bacilli for long periods of time, and, at *post-mortem* examination, show but little evidence of tuberculosis. . . . Cases have been recorded in which the only lesions found were confined to the retropharyngeal lymph glands." Gofton (1921) records that he had a cow under observation for a period of three months, during which time she continuously secreted tubercle bacilli in her milk, and yet never revealed any abnormality in the udder. Neither was it ever possible to demonstrate the organisms by microscopic examination.

Although such cases are possibly not very common, it is probable that many positive biological results may be attributed to this cause when the origin of infection cannot be traced. Furthermore, as such organisms may be excreted intermittently, they may also account for some of the so-called "inconsistent" biological results in which one sample is returned positive and a subsequent one negative.

Intermittent contamination of milk may also occur from human sources, and, judging from the number of recorded cases, it is an avenue of contamination which should always be borne in mind when difficulty occurs in identifying the source of infection.

It is not unlikely also that intermittent contamination of milk may occasionally occur from infected bovine fæces, or some discharge from an "open" lesion. According to Stenhouse Williams (1928), approximately 1·53 per cent. of apparently healthy cows pass tubercle bacilli in their fæces. Apart, however, from affording an explanation of occasional discrepant results, the contamination of milk after it leaves the mammary gland is of minor importance as compared with infection from within the gland. The reasons for saying this are, firstly, that such infection is only occasional or intermittent, and, secondly, that the number of organisms which gain entrance in this way are few. Furthermore, such contamination is only likely to be present in bulk samples. So far as properly drawn direct samples are concerned, acid-fast bacilli must be interpreted as having come from within the gland.

Mycobacterium tuberculosis (avian).

This organism is the cause of tuberculosis in birds, and about 30 per cent. of localised tuberculosis in swine. In cattle, the organism, under experimental conditions, is incapable of setting up progressive tuberculosis and there are few records of spontaneous infection.

It thus appears that avian tubercle bacilli are not likely to cause confusion by their presence in milk, as they are only likely to gain entrance when opportunity for gross contamination with poultry or pig excreta is afforded.

Although some continental workers consider man susceptible to infection, this view is not held in this country. In neither the English nor the Scottish investigations into human tuberculosis was this strain isolated, although over two thousand cases were investigated.

Mycobacterium paratuberculosis.

The causative organism of Johne's disease will only be encountered in milk when opportunities for manurial contamination have been afforded. Whilst its presence in milk is probably of but rare occurrence, its significance is purely one of hygiene as the disease to which it gives rise is peculiar to ruminants, the organism being non-pathogenic to man.

Mycobacterium lepræ.

This organism is now recognised as the cause of leprosy, but is of no significance in connection with milk, as the chances of its being encountered are very remote. It is not alcohol-fast, and attempts to communicate the disease to small laboratory animals have failed.

The following are the chief saprophytic acid-fast bacilli.

Mycobacterium smegmatis—found on the skin and genitalia of man and animals.
Mycobacterium butyricum (Petri) found in butter.
Mycobacterium berolinensis (Rabinowitsch) found in butter.
Mycobacterium friburgensis (Moeller) found in butter.
Mycobacterium stercusis (Moeller) found in manure.
Mycobacterium phlei (Moeller) found on grain.
Mycobacterium alluvialum (Kersten) found in soil.

I do not propose to consider these species individually, as comparatively little is known about them, and such information as we possess is of minor importance, and has little relation to their significance in milk. The outstanding factors concerning them are their ubiquitous distribution in nature, their acid-fast properties, the ease with which they can be cultivated, and the absence of, or their feeble, pathogenicity to laboratory animals.

The possibility of their being found in milk varies directly as opportunities are afforded for contamination. They are to be found in smegma, butter, grass, manure, hay, grain and soil. There is also the possibility, which must not be overlooked, that such saprophytes may, as in the case of other non-pathogenic species, enter the udder *via* the teat duct, and proliferate there for a time without producing detectable lesions. In such circumstances, they may appear in the milk of a healthy cow, or even also in the milk of a cow with tuberculous mastitis.

Having briefly reviewed the main features of the species of the acid-fast genus, it will be appreciated that one member, *M. tuberculosis* (bovine), is of outstanding importance on account of the frequency with which it is encountered in milk and its pathogenicity to man. The human strain, which is morphologically indistinguishable from the bovine form, may also occasionally be found.

The presence of the causal agents of Johne's disease and avian tuberculosis is so rare a possibility as to merit little consideration. Furthermore, they are only likely to be found following gross contamination with excreta, and so should never be encountered in a direct sample for microscopic examination. As far as the biological test is concerned, they are but feebly pathogenic, or non-pathogenic, for the guinea-pig, and are of no consequence.

With regard to the saprophytic acid-fast bacilli, they must always be considered as potential contaminators of milk, and, under certain circumstances, may cause confusion in diagnosis. The likelihood of error, however, is, I think, extremely small, and so long as due care and diligence are exercised in performing the tests the possibilities of a mistaken diagnosis are comparatively rare.

It is claimed, however, by some engaged in routine clinical examinations that the results of biological tests, etc., are so markedly at variance with the clinical findings that there must be some factor, other than the personal one, giving rise to these so-called inconsistent results. It is in this connection that the saprophytic acid-fast bacilli are suspected, and it is with this in mind that I propose now to consider, as far as space will allow, the possibility of such organsims vitiating the reliability of routine examinations and tests.

Such confusion may occur in microscopical examinations of stained sediment from centrifugalised individual milk samples or in the course of biological examinations. The microscopic examination of mixed samples is not often practised as, apart from the uncertainty of finding tubercle bacilli in a sample containing the milk of many cows, negative results are valueless.

(1) Microscopical Examinations of Milk Sediment

In considering the value of microscopical examinations of milk sediment it must first of all be borne in mind that the majority of samples submitted to examination are collected either as a result of some clinical manifestation of mastitis, or in order to determine which of a series of cows is giving tuberculous milk, the combined milk having proved positive to the biological test. Furthermore, the sample is usually collected by a veterinarian, and in many cases, he is the person whose duty it is to examine it microscopically.

This fact is a very significant one in the interpretation of results, as, on the one hand, the microscopist has clinical evidence to support his findings, and, on the other, he has the satisfaction of knowing that there is no possibility of extraneous contamination. This is an advantage which is denied to the laboratory investigator, whose reports are, of necessity, less definite.

In such examinations a search is made for the presence of acid-fast or acid- and alcohol-fast bacilli presenting what experience has taught us to regard as the typical morphological appearance and arrangement of tubercle bacilli. In milk sediment, tubercle bacilli appear as slender, rod-shaped organisms varying in length, but usually about 2 to 5μ long by 0·3 to 0·5μ broad. They occur singly or in groups, lying either parallel or forming an acute or obtuse angle to each other. They are usually free, but may sometimes be seen in the cytoplasm of certain phagocytic cells. Individual organisms are generally curved, but are sometimes bent at an angle. Some take the stain uniformly, particularly the shorter forms, whereas others display a characteristic beaded appearance. There are no reliable morphological differences between the various strains of tubercle bacilli as they appear in milk.

If milk samples are properly collected, one is entitled to assume that all acid-fast bacilli which are present in the milk have come from inside the body. As there is at present no justification for assuming that acid-fast bacilli other than tubercle bacilli flourish naturally in the cow's udder (except in such instances as I have mentioned, which are so rare that they can be considered negligible) there is no reason to doubt that acid-fast bacilli which in their morphology and arrangement are typical, are other than tubercle bacilli. Care, however, should always be exercised if only one or two organisms are present, or if any deviation from the typical appearance should be presented.

Thanks to Torrance (1927), who first noted their presence, we frequently have at our disposal in such examinations of milk sediment other confirmatory evidence in the form of "cell-groups." It has been shown by him and confirmed by Matthews (1931) and others that the sediments of tuberculous milk samples contain, with remarkable regularity, aggregations of cells, which are so typical in their appearance as to form very valuable corroborative evidence. Some even go so far as to say that their presence alone is diagnostic of tuberculous mastitis. I do not propose, however, to discuss their presence or significance further than to say that they have, in practice, proved helpful in such examinations. It is necessary to point out that such cell-groups are only to be found in milk accompanied by tuberculous mastitis, and that their absence is no certain indication of the absence of tubercle bacilli in milk. Tubercle bacilli originating in the system and passing through a healthy udder would not be accompanied by such cytological changes; neither, of course, will they be present when tubercle bacilli enter the milk from other sources.

Up to the present I have confined my attention to the recognised members of the Genus *Mycobacterium* or the acid-fast group proper, and it would appear that, provided the necessary precautions are taken, the only acid-fast bacilli which are likely to be encountered in milk are tubercle bacilli. This, however, does not appear to be the experience of some veterinary officers. From a series of enquiries which I have made I find that whilst the majority are of the opinion that acid-fast bacilli other than tubercle bacilli are extremely rare, others say that they may occur in as many as 5 per cent. of cases. All, however agree that there is rarely any difficulty in distinguishing them from tubercle bacilli.

Although I have no personal experience of acid-fast bacilli other than tubercle bacilli in milk sediment, I have had the privilege of examining a number of slide-preparations sent to me by veterinary officers, and I am inclined to the view that many of the organisms which are described as acid-fast are not members of the recognised group, *i.e.*, Genus *Mycobacterium*, but are organisms which, by virtue of the fact that they possess a slight degree of acid resistance, appear to be acid-fast when decolorisation has not been effectively carried out.

In stating this, I have in mind one organism in particular, namely, one which is shorter and thicker than the tubercle bacillus, and appears in groups in well-defined, circumscribed, clear or faintly pink spaces in the film, although single forms may also occur throughout the field. This organism, which appears to be diphtheroid in type, is not acid- or alcohol-fast in culture, but, in milk, I have found that, unless decolorisation is effectively carried out, many tend to retain the stain. Even when a few retain the pink colour others are to be seen stained with the counter-stain. It is well-known that some organisms which are normally not acid-fast display weak acid-resisting properties when grown in a fatty medium, and it is possible that a similar explanation may apply in this case. Judging from the number of milk samples received containing this organism, one is entitled to assume that it is responsible for a chronic insidious form of mastitis which may be confused clinically with early cases of tuberculous mastitis.

From the point of view of causing confusion in microscopical diagnosis, neither this, nor any other acid-fast organism which I have seen in milk-films, be it a recognised acid-fast member, or not, should cause much difficulty as, added to the distinguishing morphological features which I have mentioned, the so-called "cell-groups" are absent. Delepine (1910) after considerable experience in such examinations, stated: "Since 1884 several thousand films have been examined

by myself or my assistants, and I have known of one instance only in which an acid-fast bacillus has been mistaken for the tubercle bacillus."

Before leaving the subject of microscopic examinations of milk, I must refer briefly to the exceptional results published by Albiston (1930) in Australia. He stated that in the course of microscopic examinations of 213 samples from 213 farms, he found that in 55 samples acid-fast bacilli other than tubercle bacilli were present. Although these samples were mixed samples, and, as such, more liable to contamination, I can offer no satisfactory explanation of such results other than to suggest that there must exist in Australia some factor of major importance which does not apply here.

Summarising the significance of acid-fast bacilli in microscopic examinations of milk sediment, I am of the opinion that, provided the milk has been collected with reasonable precautions, and that decolorisation has been efficiently carried out, using both acid and alcohol, the finding of acid-alcohol resisting bacilli displaying the characteristic appearances and arrangement which I have described is strongly indicative of tuberculous infection. If such findings are supported by clinical evidence and the presence of cell-groups, then the results may, for all practical purposes, be taken as conclusive.

(2) The Biological Test.

The second way in which acid-fast bacilli other than tubercle bacilli may cause confusion is by the production of lesions in the inoculated guinea-pig, which bear some resemblance to those produced by tubercle bacilli. This is a possibility which I consider to be of rare occurrence.

It is important at the outset to realise that milk samples which are used for guinea-pig inoculation may be either bulk-samples, herd samples, or individual (direct) samples. In mixed samples, contamination is always much more marked, and organisms may enter the milk which do not get an opportunity of doing so in a direct sample. For example, it is nothing unusual to be able to filter out from mixed samples pieces of hay, straw, and other material. In such circumstances, one would not be surprised to meet with *Mycobacterium phlei* (the timothy grass bacillus) and other organisms. For this reason it is necessary that our test should be a very reliable one in order to discriminate between the various species.

The guinea-pig, which is the animal used in the biological test, has proved itself to be a very valuable means of diagnosing tuberculosis. By its means we are able to detect sources of tuberculous infection which are beyond the scope of the clinical-microscopic method. I refer to tuberculous milk which is not associated with clinical tuberculous mastitis, and which, on that account, is never suspected, and consequently, not submitted to examination.

In general, it may be said that guinea-pigs are only susceptible to those acid-fast bacilli which are pathogenic to man. They are very susceptible to infection with tubercle bacilli of mammalian origin, particularly to the bovine strain, but are resistant to avian bacilli. Following subcutaneous inoculation with tuberculous milk, a localised lesion forms, from which infection extends to the neighbouring lymphatic glands in which progressive caseo-necrotic lesions are produced. The most advanced lesions are situated nearest to the point of infection, and may be evident between the third and sixth weeks. Next to the glands, the lesions are most marked in the spleen and liver. It must be realised, however, that the extent of the lesions depends on the number, virulence, and strain of the organisms introduced. Whereas it is stated that one organism is sufficient to set up infection, in such conditions one could hardly expect to find extensive lesions in six to eight weeks. Generally speaking, however, tubercle bacilli are much more numerous, and are in sufficient numbers to produce lesions of macroscopic dimensions in glands and organs other than at the site of inoculation. Doubtless, more accurate results would follow the microscopic examination of smears or sections of the inguinal glands following thigh inoculation when visible lesions are absent; similar results would also follow an increase in the length of time allowed for diagnosis, and the multiplication of guinea-pigs used for each test. These alterations, however, would, to a large extent, detract from the value of the test, without making any very appreciable change in the percentage results.

As far as the present state of our knowledge goes, acid-fast bacilli other than the bovine and human strains of tubercle bacilli are either feebly pathogenic, or non-pathogenic to guinea-pigs. Although most of them are referred to as saprophytic, many of them, on inoculation, do produce localised lesions, but only under certain conditions, and this is one of the points which I wish to emphasise, and make perfectly clear, as the possibility of such lesions causing confusion appears to have shaken the confidence of a number of workers in the recognised biological test for tubercle bacilli. It is stated by some that the biological test sometimes returns samples as positive which are directly contrary to clinical and microscopic findings, and that the lesions in the guinea-pigs must have been produced by other acid-fast bacilli.

Numerous workers, in their researches with saprophytic acid-fast organisms, have shown that, when such organisms are inoculated into laboratory animals in a concentrated form, *i.e.*, in the form of cultures, they can produce lesions, but these are usually of a local character, although occasionally of a nature resembling those caused by tubercle bacilli, and even causing death. In some instances, it has been necessary to inoculate

along with these cultures other substances in the form of butter or oil before such lesions could be demonstrated. This is only what might be expected, as it is not unreasonable to suppose that massive doses of the most benign organism will, if introduced into the living body, produce some reaction; in fact, it has been shewn by independent investigators that dead tubercle bacilli when introduced in sufficient numbers will produce tubercle-like nodules (Muir and Ritchie, 1927, Hagan and Levine, 1932).

Taking into consideration the fact that these results have only followed the injection of cultures, and that even in such instances tubercle-like lesions were only very occasionally produced, it is very unlikely that such results will follow the inoculation of a milk sample collected under reasonable conditions, even should similar organisms be present.

It thus appears that the biological test is as delicate and reliable as any test can be into which the element of chance and the personal factor enter. Wilson and Nutt (1926), having examined 488 samples of milk by the biological test, came to the following conclusion: "if in the lesions in a milk-injected guinea-pig acid-fast bacilli are found, the milk contained tubercle bacilli." This is a view which is shared by workers in this country.

Albiston (1930), however, claims that "saprophytic acid-fast bacilli have been found responsible for the production of lesions in milk-injected guinea-pigs in a comparatively high percentage of cases" and out of a total number of 1,283 farm samples examined, saprophytic acid-fast bacilli were detected in 119 samples, or 9·3 per cent. In the majority of cases, the lesion was a local one involving the subcutaneous or muscle tissue on the medial aspect of the inoculated leg, but in some instances the precrural, the inguinal and the iliac glands were involved. "In no case was the infection found extending to the internal organs." To find saprophytic acid-fast bacilli in the lesions is unusual, but their presence in 9·3 per cent. of cases is unique, and to my knowledge, he is the only worker who has recorded such results with injected milk. In an effort to confirm or refute these findings, the department in which I have the privilege of working has been engaged for some time past in making a close examination of guinea-pig lesions which in any way suggest the possibility of a causal agent other than tubercle bacilli. In carrying out this investigation, tissues are collected from all guinea-pigs which present lesions other than of a progressive nature, but which contain acid-fast bacilli, and these tissues are injected into other animals to test their pathogenicity. Up to the present, all such injections have been followed by extensive lesions of tuberculosis within a comparatively short space of time. Out of a total of approximately 4,000 milk samples injected into guinea-pigs, with the exception of one case in which the inoculated animal died on the ninth day, we have not encountered a single case which has given rise to any doubt. These results, taken in conjunction with opinions expressed by other workers, seem to rule out, as far as this country is concerned, any very great margin of error arising from the presence of the so-called saprophytic acid-fast bacilli in milk, particularly if diagnoses are based on finding typical acid-fast organisms in lesions other than at the site of inoculation.

If these submissions are correct, then how does one account for:—

(1) positive biological results occurring when no source of infection can be traced;

(2) negative biological results following the injection of milk from a herd known to contain a tuberculous cow.

With respect to the first, I think the answer is to be found either in the fact that tubercle bacilli may be excreted in the milk of a tuberculous cow with a healthy, or apparently healthy, udder or in the presence of contamination from other sources. In both instances, particularly the latter, the presence of infection is likely to be intermittent.

The second charge may be explained in one or two ways. It is possible that such results are due to the fact that neither milk nor resuspended milk sediment contains tubercle bacilli in even suspension. One does not require much experience of *post-mortem* examinations to realise that the number of organisms from one sample introduced into each of two guinea-pigs often varies a great deal. It may vary to the extent that one guinea-pig is macroscopically negative, whereas the other shows extensive lesions. If, however, a short time after inoculation, the guinea-pig which has received the bulk of the organisms dies from intercurrent infection, and only the one which has received a minimum or no infection lives, what is the result? The probability is that when the time comes for it to be sacrificed, the lesions, if present, may be so small as to be overlooked. This is only one explanation; there are others, amongst which, of course, is individual susceptibility, but space will not permit me to discuss them. These explanations, however, may serve to convince you that some of the blame which is attributed to acid-fast bacilli should be transferred to other more feasible causes.

In conclusion, it appears that, provided the microscopical and biological tests are carefully carried out and interpreted in the light of modern knowledge, the liability of error is small. This varies in direct proportion to the skill and diligence of the investigator.

. . . . . .

(b) The Significance of *Bacillus coli* in Milk

In relation to milk, the term *Bacillus coli* is used in a generic sense to indicate a group of

organisms, also known as the coli group, which in their morphology, cultural characters and biochemical reactions bear a very close resemblance to *Bacillus coli communis* (*Escherichia coli*). The members of this group are invariably present in the fæces of man and animals.

For many years, the determination of the number of *B. coli* in a sample of water has been the accepted bacteriological method of ascertaining the potability of water for human consumption. It is not because such organisms are considered pathogenic, but because their presence in water in considerable numbers indicates fæcal contamination.

On the same principle it has been assumed that if such organisms could be shown to be present in milk they would form a valuable indication of the cleanliness of the methods used in its production, with particular reference to contamination by fæces. Accordingly, bacteriological examinations of milk for *B. coli* have become a routine measure and such an examination has been adopted by the Ministry of Health as one of the standards governing the production of Certified and Grade A milks.

The routine tests devised to detect *B. coli* are based on the ability of members of the group to ferment lactose with the production of acid and gas. If when a specified quantity of milk is inoculated into a tube of bile-salt lactose peptone water it shows, after 48 hours' incubation, evidence of acid and gas production, the milk is stated to contain "presumptive *B. coli*" or coliform organisms. They are called presumptive *B. coli* because the test only serves to indicate what is presumed may be *B. coli communis*, but which may be one of its congeners.

A systematic bacteriological examination of such presumptive coliform organisms found in milk has shown that they consist of two main types, the *Bacillus coli communis* or "typical" *B. coli* type, and the *Bacillus lactis ærogenes* or "atypical" *B. coli* type. Although these two types are distinguishable by their biochemical reactions, they are, from the point of view of the "presumptive test," indistinguishable and it is for this reason that the coli group is so frequently spoken of as the colon-ærogenes group.

Inasmuch as the presumptive coli test, as used in routine bacteriological examinations, responds to both the typical *B. coli* and ærogenes type of organisms, it is important in assessing the significance of the results that we should know the sources of such organisms and the circumstances under which they gain entrance to milk. If it could be shown that all presumptive *B. coli* originate from fæces, then their presence in milk could be interpreted as a measure of fæcal contamination and the desirability of identifying the precise nature of the organisms would be unnecessary.

Do Presumptive *B. coli* in Milk Indicate Fæcal Contamination?

It is generally recognised that the *B. coli communis* type occurs in enormous numbers in the alimentary contents and fæces of man and most animals, and accumulation of evidence goes to show that this is its common habitat, and the source of its distribution. It is so rarely found to lead a saprophytic existence outside the body that its presence in water or milk has come to be regarded as clear proof of manurial contamination.

With regard to the *B. lactis ærogenes* type, on the other hand, it has been conclusively shown that, whilst they do occur in fæces in small numbers, their main source is soil, cereals and vegetation.

Tonney and Noble (1931), having examined 521 samples of cattle fæces, found the relative percentage of coliform organisms present to be *B. coli communis* type 99·3 per cent. and *B. lactis ærogenes* type 0·7 per cent. They also found that *B. lactis ærogenes* predominated in soils and vegetation, the relative proportion being *B. lactis ærogenes* type 95·4 per cent. and *B. coli communis* type 4·6 per cent.

From these figures it is evident that fæces do not form an important source of the ærogenes type of organisms and thus their presence in milk must be interpreted as indicating some source other than fæcal contamination. The fact that they cannot be distinguished from typical *B. coli* by the presumptive test also means that the coliform organisms so identified cannot be regarded as certain indicators of fæcal contamination.

This suggests that there are other ways in which milk may become contaminated by colon-ærogenes organisms. That this is so there is no doubt, as, apart from our knowledge of the sources of ærogenes organisms, it is often found in practice that milk, which has been produced under conditions in which all reasonable precautions are taken to exclude fæcal contamination, may still reveal a high colon-ærogenes content, and such a count may even prove to be higher than that of a milk with which such precautions are not taken.

I now propose to consider very briefly the chief causes of high colon-ærogenes counts in milk, paying particular attention to the relative significance of such factors in relation to milk produced under reasonably good conditions.

Assuming for the present that milk as it leaves the udder of the cow is free from coliform bacteria, it follows that all organisms of this type found in milk must have resulted either from contamination during the process of milking and its subsequent handling or they are the descendants of those so introduced. Each of these possibilities will be considered in turn.

1.—*Contamination of Milk During its Production and Subsequent Handling.*

Before proceeding to the consideration of this question, it is important to realise that it is almost impossible to produce milk commercially, even under the very best of conditions, without some measure of contamination by members of the colon-ærogenes group. Samples of certified milk, more often than not, contain coliform organisms when 5 cubic centimetre quantities are tested, but if reasonable precautions are taken in its production it should rarely be possible to demonstrate them in 1 cubic centimetre of milk.

The two chief forms of milk contamination are by fæces and unsterilised utensils. Fæcal contamination may take place either directly from the body of the cow or indirectly by contaminating the utensils employed for the collection of milk. Unsterilised utensils may introduce into the milk organisms of fæcal origin or such as have entered from the surrounding atmosphere or other sources. It is not only the number of organisms introduced into the utensils which is of importance, but the fact that by continuous growth in such vessels their numbers may be countless when the utensils are next used for milking. Direct contamination of milk from the surrounding dust-laden atmosphere is of minor consequence, from the numerical aspect of organisms introduced, if one excludes large particles of fæces which enter the milk from the body of the cow. Dust contamination of utensils, which are left standing about in the cow-shed and not subsequently sterilised, is of much greater importance in view of the growth of the organisms which gain entrance in this way.

The relative significance of the above forms of contamination has been very clearly demonstrated by the exhaustive work of Ayres and Clemmer (1918) conducted under the auspices of the United States Department of Agriculture. The numerous experiments which they performed have shown that, under average conditions of cleanliness and where steam sterilisation of utensils is carried out, the number of colon-ærogenes organisms which are found in fresh milk is remarkably low. They found that approximately only 6·9 per cent. of samples so produced revealed coliform organisms in 1/100 c.c. of milk. By working with the same animals, but varying the conditions of production to suit the purpose of the experiments, the above authors found also that when the cows and cow-shed were allowed to remain dirty, the utensils continuing to be steam-sterilised, the number of samples containing coliform organisms in 1/100 c.c. of milk went up from 6·9 per cent. to 10·7 per cent. When, however, steam sterilisation was replaced by washing the utensils in hot water containing washing powder, rinsing in clean, cold water, and allowing them to stand inverted until the next milking, the number containing coliform organisms went up to 28·6 per cent., and when such washing was not performed until eight hours after milking the samples containing coliform organisms numbered 71·4 per cent.

The above findings demonstrate very forcibly the outstanding part which is played by efficient steam sterilisation of utensils. Whilst cleanliness of cattle and their surroundings does make a difference to the counts, it is not so marked as one might be led to expect, as, even under the dirtiest of conditions, the number of organisms which gain entrance into the milk does not compare with the number admitted through unclean utensils. It is important to note also that when the cattle and surroundings were left dirty and the utensils washed and not sterilised after use, over 70 per cent. of the samples satisfied the coliform requirements of Grade A milk when such milk was tested shortly after collection. It thus appears that whilst contamination by unsterilised utensils and fæcal pollution are mainly responsible for the introduction of coliform organisms into milk, it is very unlikely, under average and reasonable methods of clean milk production, that such contamination can be solely held responsible for a high *B. coli* content of milk.

2. *The Growth in Milk of Organisms Introduced during Production.*

It has already been pointed out that under commercial conditions it is impracticable to produce milk free from coli contamination. It has also been mentioned that sources of contamination other than from utensils and fæces are comparatively unimportant if regard is paid only to the number of organisms so introduced. If, however, such organisms, no matter how few they may be, are capable of growing in milk, it is obvious that unless something is known of the conditions under which the milk has been produced the coli-presumptive test can be of little value in interpreting either the nature or degree of contamination.

This being the case, it is of the utmost importance that one should know if growth can take place, and, if so, to what extent it may influence the colon-ærogenes count.

Tests have shown that little or no growth takes place when milk is kept at 50° F. (10° C.) or under, but as the temperature increases above that point, rapid multiplication of the organisms takes place which is directly proporfional to the conditions of temperature and time to which the milk is subjected. An idea of the significance of these conditions can be obtained by keeping samples of Grade A milk at a temperature of 70° F. (21° C.) for 24 hours. It has been shown that such samples, containing no coliform organisms in 1/100 cubic centimetre when fresh, may contain millions of coliform organisms at the end of 24 hours, and yet if the milk is kept at 50° F. (10° C.) or under, little or no growth takes place.

It thus appears that the temperature at which milk is kept subsequent to production has a very important bearing on the resulting coli content of milk. Furthermore, a comparison of the numbers of organisms which may enter the milk when produced under dirty conditions with the numbers which result from keeping milk at a temperature conducive to bacterial growth, suggests that the temperature factor is of much greater importance from the numerical aspect than any condition of cleanliness observed during production. Milk which has been produced under the cleanest conditions possible, but which has been maintained at a temperature favourable to bacterial growth may reveal a much higher coli content than milk produced under dirty conditions but which has been kept below 50° F. (10° C.). It is this temperature question which constitutes the almost insuperable difficulty of milk producers and distributors who have to depend on the present ordinary methods of transport for the conveyance of milk during warm weather.

Up to the present it has been assumed that all coliform organisms which are found in milk are the result of contamination in the collection and handling of milk. Whilst the exceptions to this statement are perhaps, for all practical purposes, so few as to be considered negligible, it is, nevertheless, necessary to bear in mind that coliform organisms may enter the udder and grow there, being excreted continuously in the milk without producing any obvious pathological change. Gofton (1931) records two such cases. It is also necessary to remember that *B. coli* have been associated with certain forms of bovine mastitis, but in such conditions it is probable that the pathological change in the udder would be sufficiently marked to cause the exclusion of the cow from the milking herd.

Conclusions

From the above facts one is entitled to conclude that whilst the presence of *Bacillus coli* in milk, as determined by the presumptive test, affords a valuable indication that the methods employed in the production or keeping of milk are at fault, the test is of no value as an indication of gross contamination, as one cannot distinguish between organisms introduced during contamination and such as have subsequently grown in the milk.

Furthermore, as the presumptive test for *B. coli* does not discriminate between *B. coli communis* of fæcal origin and *B. lactis ærogenes* of non-fæcal origin, the test affords no indication as to the nature of the contamination.

Presumptive *B. coli* in milk usually result from contamination through unsterilised utensils, fæcal contamination, or the growth of organisms introduced in these ways.

A high *B. coli* content of freshly-produced milk indicates faulty production and usually the use of unsterilised utensils.

A high *B. coli* content of milk (at the time of delivery to the consumer) which has been produced under reasonably clean conditions can nearly always be attributed to the keeping of milk at a temperature favourable to the growth of bacteria.

References

Albiston, H. E. (1930). *Austral. Vet. J., 6,* 123.

Arkwright, J. A. (1930). "A System of Bacteriology," *1,* 317. H.M.S.O., London.

Ayers and Clemmer. (1918). U.S. Dept. of Agric. Bulletin, No. 739.

Delepine, S. A. (1910). Quoted from Savage, W. G. (1929). "The Prevention of Tuberculosis," p. 118. Macmillan and Co., London.

Gofton, A. (1921). *Vet. Rec., 33,* 287.

Gofton, A. (1931). Proc. 11th Internat. Vet. Congress, 2, 310.

Griffith, A. S. (1911). Royal Commission on Tuberculosis. Final Rep., Appendix *111,* 79.

Hagan and Levine. (1932). *J. Amer. Vet. Med. Ass., 81,* 723.

Matthews, H. T. (1931). *Vet. Rec., 11,* 403.

Miller, F. R. (1932). *J. Exp. Med., 56,* 411-424.

Muir and Ritchie. (1927). *Manual of Bacteriology,* p. 332. Oxford University Press.

Stenhouse Williams. (1928). *Vet. Rec., 8,* 293.

Tonney and Noble. (1931). *J. Infect. Dis., 48,* 413.

Torrance, H. L. (1927). *Vet. Rec., 7,* 875.

Watson, E. A. (1931). *Proc. Internat. Ass. Dairy & Milk Insp.* (1931.)

Weber and Titze. (1907). Cited by Cobbett (1917). "The Causes of Tuberculosis." Cambridge University Press.

Wilson and Nutt. (1926). *J. Path & Bact., 29,* 147-148.

Discussion

There was again a crowded attendance at the Town Hall, Llandudno, at the earlier session on the morning of Tuesday, September 19th, when the first meeting of the Public Health Section was held, with Lieut.-Colonel J. A. Dixon, T.D., M.R.C.V.S., in the Chair, in the absence of the President of the Section, Lieut.-Colonel J. W. Brittlebank, and of Major D. S. Rabagliati (Vice-President).

The Chairman introduced Mr. Davies, the writer of the paper, who intimated that he did not desire to amplify his communication as circulated.

The discussion was opened by Captain H. L. Torrance, M.R.C.V.S., D.V.S.M., Chief Veterinary Officer, Nottinghamshire, in the following terms:—

I am sure we are all very much indebted to Mr. Davies for presenting us with such an excellent paper containing so much original work and thought on a subject about which we still have a great deal to learn.

He has gone into the matter most thoroughly, and has elucidated some very important points which will be of great practical value, particularly to those more directly concerned with the

protection of the public from the dangers of tubercle infection in milk.

As Mr. Davies spends most of his time in the laboratory, while my time is spent very largely in the examination of dairy cows, I trust you will make allowance for my shortcomings in opening the discussion on a paper by a master of a subject concerning which I possess but a smattering of knowledge.

The paper contains two definite sections—The Significance of (*a*) Acid-fast Bacilli, and (*b*) *Bacillus coli*, in Milk—and I propose, with your permission, Sir, to devote most of the time at my disposal to discussing the former section because it concerns us all as veterinary surgeons, and especially those engaged in work under the Milk and Dairies Acts and Orders, and the Tuberculosis Order of 1925. It is almost entirely a veterinary matter, as most acid-fast bacilli are found in milk samples taken from cows suspected of suffering from tuberculous mastitis and sometimes cause confusion in the diagnosis of that condition, whereas the coliform bacillus, which is quite commonly a contaminant from dairy utensils, seems to be a cause of tremendous alarm to all those in any way concerned with milk except, happily, the unwitting consumer.

Bacillus Coli in Milk

In dealing with the *Bacillus coli* Section of the paper, it is worthy of note that recent investigations have shown that our dear old friend—the cow—is not nearly so black as she is often painted. At one time, you remember, not so many years ago, if a milk was found to be heavily infected with coliform organisms there was considered to be just one explanation, namely—bovine fæces. It has been extremely interesting to note the gradual change of thought leading up to the quite modern one so ably expressed in Mr. Davies' paper.

When milk is produced, the number of *B. coli* present will depend on the degree of cleanliness practised during the process, and the nearer that approximates to a surgical operation, the cleaner it will be, but, as practised under the best conditions there are always some *coli* present. Now the number of coliform organisms present in any given milk by the time it reaches the consumer will depend on the number present when it left the cowshed, the number of contaminants added from dairy utensils, air, etc., and the temperature of the milk from the time it leaves the cow until it reaches the consumer. In the investigation as to the source of contamination in a "dirty" milk, these factors should all be enquired into in the interests of truth and equity, although there seems to be a tendency still to blame the producer much oftener than is justified. The numbers of *B. coli* found in pasteurised milk, and the frequency with which they are discovered, has had a very salutary effect in indicating a not by any means uncommon source of contamination, and has so relieved to some extent the burden usually placed on the producer.

The need for scrupulous cleanliness in the production of milk is obvious to all, and yet I am sure there is not a person in this room who has not been wearied almost to slumber by some enthusiast recounting the enormous number of things one should do, to be exceeded only by the enormous number of things one should not do, in the production of clean milk.

I do not wish to detract in any way from the need for thorough cleanliness in the production of milk, but having produced that invaluable article, I wish to emphasise the necessity for keeping it clean, by seeing that it is put in properly sterilised containers, and that it is transported and stored under suitable conditions, especially in relation to temperature.

Acid-Fast Bacilli in Milk.

Any person who has been engaged in dairy inspection for any length of time must have been struck with the extraordinary discrepancies occasionally met with between reports on the biological examination of milk samples and the results of the clinical examination of the dairy cows concerned. What are the common reasons given to explain these mysteries? The more important ones usually put forward are, I think:—

1. That tubercle bacilli can pass through the normal healthy udder into the milk.
2. That some cows pass tubercle bacilli in their milk intermittently.
3. That the tubercle bacilli reach the milk from some extraneous source, *e.g.*, fæces, urine, expectorate, etc.
4. The possibility of acid-fast bacilli, other than tubercle, producing lesions resembling tubercle in the inoculated guinea-pig.
5. Changes in the members comprising the dairy herd.
6. Infection from a human source gaining access to the milk.
7. The possibility of human error.

1. *Tubercle Bacilli passing through Healthy Udder into Milk.*—With regard to this, I simply do not believe that such occurs. It is far too big a subject to discuss here, but in the time I have I will give you a few particlars and figures to show that if it does occur at all it happens so seldom as to be negligible. In Nottinghamshire, out of 44,406 cows whose milk was examined biologically, 229 were found with definite clinical lesions in the udder, and one cow was found passing tubercle bacilli in her milk with no clinical evidence in the udder. Let us take the number of cows as approximatey 44,000 and that 50 per cent. would be tuberculous in some degree, then out of 22,000 tuberculous cows

whose milk was examined biologically only one cow showing no clinical udder lesion was found to be passing tubercle bacilli in her milk. The udder of this particular cow was sent to a Pathological Institute for examination for the presence of tuberculous mastitis, but no evidence of tubercle was found microscopically or biologically. I need not point out, however, that that does not mean that there was no lesion of tubercle in the udder. In my opinion, there must have been a lesion in the udder somewhere, because on two occasions when I examined the milk sediment microscopically I found cell groups and acid-fast bacilli which I thought were tubercle, two on the first examination and one on the second.

Mr. Davies deals with this subject in his paper, and says that it "appears to be established that bacilli may pass from the system into the milk through the healthy udder." I am not so satisfied as Mr. Davies that it has been established, and even if it has, my figures show that such an occurrence, if it exists at all, is extremely rare. I wish to emphasise this point particularly because I think it is a great mistake to alarm the public unnecessarily about the dangers of milk, and by so doing render a disservice to the milk producer who, after all, is doing a great deal to supply the public with a wholesome article.

2. *Cows passing Tubercle Bacilli in their Milk Intermittently.*—I suppose this statement presupposes a tuberculous lesion in some part of the udder. So far as I am concerned, it must, because I do not believe that tubercle bacilli can, under natural conditions, pass through the normal healthy udder into the milk. If we have a small tubercle or tubercles in the udder from which tubercle bacilli are passing into the milk, in order that the bacilli may appear in the milk intermittently the lesion or lesions would have to "close" and then "open" alternatively. Is this at all probable? I submit that it is extremely unlikely, if it even happens at all, and then only in the case of a dry pregnant cow, and that it will only be a "closed" lesion once, and that for a very short time after the cow has calved. It is theoretically possible for a cow to develop slight udder tubercle, and pass the infection in her milk towards the end of her lactation period, and that when the cow is dry, the udder "resting" (and her defensive forces, especially locally, are at their best during this period) the lesion or lesions may become surrounded by connective tissue and the tubercle bacilli completely enclosed, and that the position may remain unchanged until the cow calves, when the lesion will again break down and the milk become re-infected with tubercle, but this time the udder lesion will become progressive.

Do cows really pass tubercle bacilli intermittently? And, if so, what actually happens when they do? I leave it to you to tell the meeting this morning.

3. *Tubercle Bacilli from an Extraneous Source.*—We all know that this does occur and frequently explains cases where a churn sample is positive, but the direct sample negative. It is easily understood how this happens, so I will not say more about it, except that in Notts. it must happen very seldom in the milk samples submitted for biological examination, as the great majority of our mixed milk samples are drawn direct from the cows.

4. *The Possibility of Acid-Fast Bacilli other than Tubercle Bacilli Producing Lesions Resembling Tubercle in the Inoculated Guinea-Pig.*—If acid-fast bacilli other than tubercle bacilli in milk can, and do, produce local lesions resembling tuberculosis in guinea-pigs in this country, as Albiston states they do in Australia, we have an explanation for some of the very puzzling discrepancies which occur from time to time. But does this actually happen in this country? So far as I am aware, no one has ever said that it does, but Albiston has found an acid-fast bacillus in milk in Australia which he states is "liable to cause confusion, especially where lesions of lymph glands are found in milk-inoculated guinea-pigs."

I must say that, after reading Albiston's article (which my good friend Mr. Pillers very kindly brought to my notice) I did feel a little apprehensive on the subject, and decided to write to Professor Gaiger, of the Department of Veterinary Pathology, Liverpool University, to ask whether he thought any acid-fast organisms occurred in this country which were likely to vitiate the result of the biological examination of milk samples for tubercle. Professor Gaiger at once tackled the problem with his customary thoroughness and energy, and in Mr. Davies' paper and an article on this subject by Gaiger and Davies that appeared in a recent issue of the *Veterinary Record,* we have, I think, quite convincing evidence that saphrophytic acid-fast organisms are not likely to cause confusion to any appreciable extent in the diagnosis of tubercle in milk-inoculated guinea-pigs in this country.

5. *Changes in Members Comprising the Dairy Herd.*

6. *Infection from a Human Source Gaining Access to the Milk.*

7. *The Possibility of Human Error.*

The explanation of many a mystery has rested quite simply in one of the above three headings, but to deal with them to-day would, I think, be a digression from the subject under discussion.

Let me just say, however, with regard to No. 7, that in the investigation of some of these baffling discrepancies it is essential that the relationship between the field and the laboratory

workers should be most cordial, helpful and sympathetic.

I am glad to see that Mr. Davies devotes quite a large section of his paper to the "Microscopical Examination of Milk Sediment," and has dealt with the subject so thoroughly, because I would like to think that we are nearly all engaged in this work to some extent, and those of us carrying out the duties under the various Acts and Orders relating to milk, to a much greater extent.

I agree whole-heartedly with Mr. Davies that the best results are likely to follow microscopical examinations carried out by the clinician who examines the cow supplying the sample. Dual responsibility is nearly always to be deprecated, and there are definite advantages why it should be avoided in this case. There is simply not anything like sufficient time at my disposal to discuss this section as thoroughly as it deserves, and while I would like to go through it in detail, mentioning all the points with which I agree and from which I differ, little benefit might result, so I have decided to be as brief as I can, and yet try to convince those who, from lack of opportunity of enthusiasm, or fear of error, have never felt the thrill of this congenial work, how easy it is.

What are the acid-fact bacilli we are likely to encounter in the microscopical examination of freshly drawn milk from a cow with a suspected tuberculous udder?

In all the examinations I have made, I have found only three, but there may be a fourth. They are as follows:—

1. The tubercle bacillus.
2. An acid-fast diphtheroid.
3. A strongly acid-fast bacillus.
4. Possibly Johne's bacilli.

1. I need not say anything about the tubercle bacillus here.

2. *The Acid-fast Diphtheroid.*—Mr. Davies deals with this organism in his paper on page 5, at the foot of column 1 and the top of column 2. I should, however, like to emphasise what Mr. Davies says about its occurrence in small areas of the slide that are clear of cells and stained faintly pink. If any are seen among the cells at all, as a rule they are quite near to the small pink spaces referred to, just as if they had strayed a short distance from the fold, so to speak. They vary a good deal in shape, are all very small, and decolourise fairly readily, so that one sometimes sees some stained red and some blue on the same slide. As a rule, they are not readily detected without a good deal of searching, which, in the examination of a film preparation for tubercle bacilli, is quite unnecessary, and consequently when one has had some little experience of this work the only acid-fast bacilli one sees, as a rule, are tubercle bacilli.

3. *A strongly Acid-fast Bacillus.*—This organism is extremely rare, is more like the tubercle bacillus, but is more slender and has not the characteristic arrangement nor such clear beading. As a rule, they are fairly numerous. The organism is very resistant to sulphuric acid, and is not decolourised by 20 minutes in 20 per cent. sulphuric acid.

4. *Possibly Johne's.*—I have found this bacillus in the supramammary lymphatic gland but have not, so far as I know, seen it in milk.

The Percentage of Slides showing Acid-fast Bacilli other than Tubercle Bacilli.—The chief factor in this connection appears to be the amount of time spent in examining the slide. I remember when first I took over my duties in Notts., and most of us were very enthusiastic about the microscopical work and some not too familiar with cell groups, we found acid-fast bacilli other than tubercle bacilli in 10 per cent. of the slides, simply because those to whom the work was new searched for longer than was necessary, but now with the experience and the knowledge they have gained concerning the significance of the cytological picture, especially cell groups, we do not find acid-fast bacilli other than tubercle in more than 3 per cent. of the milks examined.

Cell Groups in Relation to the above-mentioned Bacilli.—Let me say straight away that the absolute secret of success in the microscopical examination of milk sediment for tubercle bacilli depends on one's ability to recognise cell groups. Generally speaking, typical cell groups are only found on a slide prepared from a tuberculous milk, so that if you find acid-fast bacilli on a slide unaccompanied by typical cell groups, one can be almost certain that they are not tubercle bacilli. This observation does not apply to the secretion from a tuberculous quarter which is literally teeming with tubercle bacilli—as a rule, cell groups are present, but they may be absent in such a secretion, which is usually like serum, but from the point of view of diagnosis they are unnecessary as the clinical evidence itself in such a case should be conclusive, but when accompanied by a secretion so rich in bacilli there can be no doubt. One wonderful feature of cell groups is that they are practically always present when the bacilli are scarce; in other words, they are present at the very time they are really helpful. Cell groups are not 100 per cent. reliable—that has never been claimed for them, at any rate by me—but they appear on slides made from the sediment of milk from tuberculous quarters with such regularity that they are of the greatest value not only in detecting tubercle bacilli, but also in distinguishing them from other acid-fast organisms.

Professor H. S. Gaiger (Liverpool), said that there were two matters which occurred to him in connection with that paper. The first was the need for establishing the reliability of the biological test. As Mr. Davies had pointed out,

doubts had been cast upon it by Albiston's work in Australia, but, so far as he knew, nothing that had been done in this country had really given cause to lead anyone to have a doubt as to the reliability of that test. He did hope that those who were engaged in carrying out the test would endeavour to prove or disprove what Albiston had said from Australia.

The next point that occurred to him was that which Mr. Davies had brought out so well, *viz.*, that the presence of *Bacillus coli* in milk was not necessarily to be taken as an evidence of fæcal contamination. That, he felt, must be news to many there; it had for years been accepted as an indication of the presence of fæcal contamination, but he thought it was brought out quite clearly that *Bacillus coli* in milk simply indicated faulty handling.

Mr. Hugh Begg (Lanark) observed that he thought, looking at the matter generally, they were in possession of many accurate facts with regard to those acid-fast organisms in the milk samples that they took from suspected cows. He wished, however, that the essayist had said something about the possibility of transmutation of one type to another, as suggested by Petroff. He had had some very disconcerting experiences in Lanarkshire which he duly recorded in his Annual Report. Edinburgh indicted the milk supply from a small herd of seven cows, a herd which he had some reason to believe was perhaps tubercle-free, from the results of the use of tuberculin in the herd two years before. His assistant went up and took individual samples from each of the seven cows, and microscopic examination proved negative. No clinical evidence of tuberculosis was found. A biological test was made with each sample. In due season five of the seven guinea-pigs were reported tuberculous. Some of the guinea-pigs died prematurely and samples had been repeated. [By the way, could some one tell them how to keep guinea-pigs alive? (Laughter.)] Re-examination of the cows revealed nothing suggestive of tuberculosis of the udder. Having dealt with these cows under the Tuberculosis Order, they found no evidence of tuberculosis on *post-mortem* examination in three cases and in the other two only the minutest lesions, none of which were in the udder. In every case Johne's disease was present, and one would like to know whether there was relationship between the acid-fast bacillus of Johne's disease and that of bovine tuberculosis. Was that experience a possible instance of transmutation of bacilli? He had sometimes had reason to doubt the reliability of the biological test, but he was not in possession of sufficient data to give proof of the wisdom of his doubt.

The diphtheroids which had been spoken of, they met with quite often. The product from the indurated quarter in these cases was of a bright flame colour that was not quite the same in appearance as the amber-coloured discharge got in an advanced case of tuberculosis of the udder. Those diphtheroids were often acid-fast, but on occasion they met with some which were not. Therefore he suggested that research workers had a great deal of investigation to do yet before they cleared the air very effectively.

Proceeding, Mr. Begg said that he did not know how the County Veterinary Inspectors could be expected to do all the microscopic examination of milk samples in addition to their ordinary work. It was the negative samples that took up time, and a large percentage of them proved negative. In his view, the opposite procedure was desirable—it was a good arrangement to have to send your samples to a man who knew nothing about the clinical features of your case. There was much virtue in having the "straight to goodness" opinion of an outside specialist. (Hear, hear.) That was the position that obtained in Lanarkshire. He never troubled the laboratory people beyond the occasional examination of a slide relating to a case that was likely to be disputed or come to court.

If the result of the microscopic examination did not tally with one's clinical opinion in a highly suspicious case and despite the fact that a biological test had been done, one should go back again, even for the fourth or fifth time and get repeat milk samples for microscopic examination.

He agreed with all those people who doubted the significance of the finding of *Bacillus coli* in milk. The importance still attached to it was a relic of a far-back fear that typhoid fever was going to be conveyed to people if they drank milk containing *Bacillus coli*. He thought they should pay little attention to the presence in milk of that organism.

Mr. W. Tweed (Sheffield) said that he would like to congratulate Mr. Davies upon his paper, and Mr. Torrance upon the informative manner in which he had opened the discussion. In his view, both had talked a lot of common sense, but what struck him as peculiar was that, as a bacteriologist and thus engaged in looking for the weak points in their tests, Mr. Davies had apparently overlooked the point that, in reality, they had no very reliable tests to work on. In regard to acid-fast bacilli other than tubercle, it had been his (Mr. Tweed's) experience many a time to get these acid-fast organisms, yet, on taking another smear and decolourising it better, more would be found. Therefore, faulty technique entered a little into the findings of acid-fast bacilli which were non-tubercle.

A point had been raised about microscopical examination being done by someone other than the man who performed the clinical examination. He thought that when one did a large number of these examinations it was best to leave the

staining to the laboratory assistant, but to do the microscopic examination oneself.

So far as the biological test was concerned, there had been no mention of a source of disease in guinea-pigs which had been investigated by Edington, *viz.*, ærtrycke infection of the gall bladder. They had had serious outbreaks of that disease, and it spoiled the examination, because it meant that the cows all had to be re-sampled. He thought that was one of the things they had to avoid, and they should be careful to purchase healthy guinea-pigs from clean sources and keep their cages clean.

Another point that arose in connection with the taking of individual samples was when you got a doubt about the acid-fast non-tuberculous organism. No man who knew his job was going to take the risk of slaughtering unless he was certain of his diagnosis. For the past twelve or 18 months he had been using the double intradermal test on guinea-pigs used for the infection of special samples. That procedure produced a result much more rapidly. But it was not to be recommended for routine testing with all guinea-pigs injected with mixed samples, because it entailed too much work; it could, however, be recommended for guinea-pigs which had been inoculated with milk from an individual cow, as a positive result could often be obtained in a fortnight in this way.

In regard to *Bacillus coli*, at the International Veterinary Congress he had said that in Sheffield he had started using that test and had disregarded the count altogether. That had been carried on in Sheffield since, and all their mixed samples were submitted to the test for *B. coli*. At first they thought the presence of this organism in the milk was due to fæcal contamination, but, like Mr. Davies, they had changed their opinion very considerably. Had it been Mr. Davies' experience that it was sometimes impossible to sub-culture from the McConkey's tubes and no growths were obtained in these cases? As a routine test for non-graded milks, to enable one to trace the source of faulty production, the coliform test in 1/100th of a c.c. would be recommended; it certainly did point to defective methods of milk production. They had now, in Sheffield, got 75 per cent. of their milk supply free from any *coli* infection in 1/100th of a c.c.

Captain R. Simpson (Carlisle) expressed the view that it was of very valuable assistance to them to have a paper on this subject, particularly when questions affecting the milk supply were the subject is of so much attention at the present time.

Mr. Davies in his paper stated that it appears to be established that tubercle bacilli may pass through the system into the milk through the healthy udder. Mr. Torrance, in opening the discussion expressed disagreement with this view. He (Mr. Simpson), was, however, inclined to disagree with the opener of the discussion, because he had had cases where tubercle bacilli were excreted in the milk without there being any discernible lesion in the udder, either on macroscopic examination or microscopic examination of the substance of the organ. He had in mind also, cases where the supra-mammary gland was affected whilst the udder substance was healthy, but where tubercle bacilli were being excreted in the milk. Such cases supported the views expressed in the paper. He believed however, that such cases were comparatively rare, as were also cases of intermittent infection of the milk with tubercle bacilli.

Mr. Begg had raised the question of the veterinary inspector or practitioner carrying out his own microscopic examination of milk for tubercle bacilli, and expressed the opinion that such examinations were best done in a laboratory. He (Mr. Simpson), entirely disagreed with this view. He felt sure that there was a great deal to be gained by the veterinary inspector carrying out his own microscopic examination of milk where he had the clinical history and picture before him to guide him in microscopic work.

One would have liked the writer of the paper to have told them exactly what he thought of the relationship of the total bacterial count in milk and the presence of *B. coli* as a guide to estimate the standard of purity of milk. Such points were of considerable importance at the present time owing to the possibility of milk being sold on a quality basis under the new Milk Marketing Scheme. It was of importance that they should have in mind some idea as to what standard should be adopted, particularly in regard to *B. coli*.

He was very grateful to Mr. Davies for his paper, and to Mr. Torrance for his excellent opening of the discussion.

Mr. W. A. Pool (Surbiton), said that a point raised by Captain Simpson appeared to him to be worthy of at least a query, *viz.*, his statement that tubercle bacilli were excreted from the udder in the absence of any lesion of that gland. To prove that the udder was free from lesions was a very laborious process. Perhaps he had misunderstood Captain Simpson, who maybe would tell them a little more fully what he meant on that point.

Captain Simpson replied that in the cases to which he had referred, the udder substance was very carefully examined and sections were cut from any portion showing the slightest abnormality. He would be glad to hear from Mr. Pool what they should do in addition, to prove that no lesion was actually present. One realised the difficulties of proving a definite and complete negative when dealing with such an organ as the udder.

Mr. Pool commented that, because one was unable to demonstrate a lesion, one was not entitled to say that none was present.

A Member observed that he had been rather surprised, when taking samples of milk from the udder in what he regarded as obvious cases of clinical tuberculosis of that gland, and even where the milk had been altered to the resemblance almost of serum, that tubercle bacilli had been very hard to find.

He, too, had found the acid-fast diphtheroids, and he had usually found that if one looked long enough one would discover some which were decolourised and some which were not.

Mr. C. E. Perry (Bristol) said that Professor Gaiger had raised the question of the reliability of the biological test. He (the speaker) as a veterinary inspector (under the Milk and Dairies Order) for Gloucestershire, could assure them that it was not 100 per cent. perfect. In Gloucestershire, the veterinary inspector had nothing to do with the carrying out of the biological test. The milk sample was taken in the usual way and sent to Bristol University, and he could give them two instances in which he was let down badly by the biological test.

In the first instance, he found a cow, in a herd, suffering from what he regarded as a typical tuberculous udder. Milk samples were taken, but were returned as negative to both the microscopical and biological tests. Seemingly secure in this knowledge, a client of his bought the cow. Some months afterwards she was killed—fat and in good condition, but she was one mass of tubercle. Tuberculosis included the udder, and through that he lost a good client.

To prove the case from the opposite angle, he would recount the second instance. Tuberculosis bacilli had been found in the milk of a herd, and he was sent to inspect it. He found what he thought was a typical case of tuberculosis of the udder; he was so sure of it that he took milk from that cow and also a bulk sample from the remainder of the herd. The bulk sample came back "negative" and the sample from the individual cow was negative both to the microscopical and biological test, although tubercle bacilli had been found in the milk previously. So sure was he of his ground, however, that he took the cow under the Tuberculosis Order, and found she was suffering from advanced tuberculosis, including the udder and supramammary glands.

He must be fair to the test, and say that in every case where the biological test had been positive, the *post-mortem* examination had confirmed the diagnosis and the udder or supramammary gland or both had been affected in every case.

Sir Weldon Dalrymple-Champneys (Ministry of Health) expressed his admiration both for the paper and for the discussion, which in common had been of extraordinary interest.

One point that rather surprised him was that anyone should expect a test of that sort (referring to the microscopical examination of milk samples) for tubercle bacilli to be anything like perfect. He had not himself conducted group examinations for the presence of tubercle bacilli in milk, but he had handled the results, and if one could take as a parallel the examination of human sputum for tubercle bacilli, one knew well that, time after time, one met with cases proved to be clinically tuberculous, in the sputum of which at times no tubercle bacilli could be found, though one felt sure that they were being excreted, at any rate, in small numbers. He imagined that the same thing happened with regard to milk.

It must be remembered, moreover, that even in the biological test they were only examining a sample of a material in which, from its nature, tubercle bacilli, if present at all, were bound to be unevenly distributed.

Considering these inherent difficulties, he thought it was an extraordinarily reliable test, in fact, much more reliable than one would expect it to be.

THE REPLY

Mr. G. O. Davies prefaced his reply to the discussion by expressing his thanks for the very generous way in which the Congress had received his paper, and his indebtedness to Mr. Torrance for the very excellent introduction which he gave. He felt that Mr. Torrance's views on the milk problem in general always commanded attention; the more one listened to him the more one realised that he never spoke without having something useful to say to them. (Hear, hear.)

Mr. Torrance's first question was as to the possibility of the intermittent excretion of tubercle bacilli in milk. It was very difficult, if indeed it were possible, to say whether or not intermittent excretion of tubercle bacilli in milk actually took place. They had no infallible test for the presence of tubercle bacilli in milk. A positive biological result certainly indicated that there were tubercle bacilli present in the milk sample, but when the test was negative it meant comparatively little, as it had been shown that guinea-pigs might occasionally fail to become infected after inoculation with tuberculous milk. The test was extremely accurate, but so long as one did encounter cases in which the test appeared to fail he did not think that they would ever be in a position to say that intermittent excretion of tubercle bacilli did, or did not, take place. In the average case of tuberculous mastitis he thought they could assume that the number of tubercle bacilli in samples of milk was reasonably constant, but in such cases where the pathological process was not so advanced

there was likely to be a greater degree of variation, and in the so-called pre-clinical stage the milk might exhibit very marked variation in the number of organisms present in each sample.

Whether tubercle bacilli could, as claimed by Watson and others, pass through the normal udder was a very difficult question to answer, seeing that, as Mr. Pool had stated, it was almost impossible to say definitely that lesions were absent in the udder. They did encounter cases, however, which lent strong support to the suggestion that such a condition might occur, and to him it did not appear an unreasonable suggestion. They knew that tubercle bacilli could pass through the mucous membrane of the alimentary canal without producing lesions—also through the lymphatic glands—therefore he thought that they might occasionally also pass through the healthy udder.

With regard to the presence of other acid-fast organisms found in milk, he came prepared to hear dissertations on acid-fast organisms of all shapes and sizes which had been encountered by various members. He had been disappointed in that; but to him it did not matter a great deal as to whether or not acid-fast organisms other than *Mycobacterium tuberculosis* were found in milk, so long as they were satisfied in their own minds that they could distinguish such bacilli from tubercle bacilli, and so long as there was no reason for suspecting that their confidence was misplaced.

The question raised by Mr. Begg with regard to the transmutation of species would call for much more explanation than he could give. It had been claimed that saprophytic organisms after long existence in the animal body might assume properties similar to the pathogenic organisms. It had been claimed by others that certain species of tubercle bacilli, *e.g.*, the avian form, after a long residence in bovines could assume the properties associated with the bovine type. It had also been put forward that the bovine strain could change in the human subject to the human form. That was a question to which the Royal Commission on Tuberculosis gave their attention, and the results of their work led to the conclusion that such cases which appeared to indicate that transmutation of species occurred, could be explained in one of two ways: either that spontaneous infection took place with the other strain after the experiment commenced, or that the artificial culture which was inoculated was mixed. The Commission had not been able to substantiate any claim for the change of one form of tubercle bacilli into another. If that were so, he thought that the possibility of Johne bacilli assuming the properties of tubercle bacilli more remote still.

Mr. Begg had also referred to the advantages of having a third person to examine milk samples. There might be advantages such as he mentioned, but he (Mr. Davies) thought they were outweighed by the distinct advantages to be gained if these examinations were conducted by the person who collected the milk samples. When laboratory people were asked to examine milk samples, they were rarely asked to do so less than 24-48 hours after collection, by which time there was a marked change in the cytological picture.

He was not prepared to go so far as Mr. Torrance, and to say that one only got cell groups in the tuberculous form of mastitis; they might get cell groups in other forms of mastitis, possibly not the typical ones, but it was necessary to bear in mind that they were only desquamated epithelial cells. He might also mention that from the laboratory aspect they rarely got milk samples which were free from other organisms (such as cocci) which were known to be proteolytic. If milk samples did not arrive for examination for 24-48 hours, it was quite likely that many of the so-called characteristic cells associated with tuberculous milk would be missing.

He was very interested in the cases quoted by Mr. Perry, because he brought them forward as an illustration of the fallibility of the biological test. As he (the speaker) had mentioned previously, he did not claim for one moment that the test was infallible; at the same time, it was, nevertheless, a very accurate and delicate test. Mr. Perry's cases did lend some support either to the possibility of the intermittent excretion of tubercle bacilli, or to the possibility that tubercle bacilli might pass through the healthy udder.

With reference to *Bacillus coli*, he did not think he had anything to add to what he had already stated in the paper. There was no doubt, in his mind, of course, that the first factor of importance to be considered when one got a high *B. coli* count was: Has the milk been kept at a temperature conducive to bacterial growth? If they could not eliminate that he did not think they need waste a great deal of time in finding out from where the *B. coli* had come. If they could eliminate that possibility, then they had to enquire into the condition of the utensils—steam sterilisation was the only effective method of getting rid of contamination from that source—and, finally, there was the possibility of contamination by fæces, direct or indirect.

According to Hastings, anthrax spores may remain viable and virulent in naturally contaminated water for 18½ years. The test was made on a sample of water that had been polluted from a tannery. This sample of water was kept in a glass stoppered bottle and subjected to the varying temperatures in a laboratory. If spores of anthrax bacilli will remain viable and virulent for 18½ years under the conditions described in Hastings' experiments, it is probable that they will live indefinitely in soil and that anthrax will become more widespread as new areas of infection are established.—*Vet. Med.*

General Articles

BLOOD-SUGAR AND BLOOD-UREA IN TRYPANOSOMIASIS

Preliminary Investigation

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The investigation was commenced in 1927, with a view to finding out whether any variations from the normal values exist in trypanosomiasis, particularly with regard to *T. vivax* and *T. congolense* cases. At that time little was known of the normal figures for the bovine, with the exception of the results obtained by the following workers: HAYDEN and SHOLL[1] gave 0·0518 per cent. for blood-sugar; WIDMARK, CARLENS and AUGER[2] gave 0·06-0·08 per cent. blood-sugar; G. et R. MOUSSU[3] 0·0616 per cent. blood-sugar; and HAYDEN[4] found 30·11 mllgm. per cent. as the normal blood-urea. All the above workers were working in connection with milk fever.

The first part of this investigation, therefore, deals with the normal blood-sugar and blood-urea values in cattle. The results obtained may be summarised as follows:—

(A) Blood-Sugar (Using McLean's Method)
Average, Normal Cattle, Uganda
(48 tests) 0·0525 per cent.
0·035 per cent. Minimum.
0·085 per cent. Maximum.

These tests were made on eleven cows, six calves, and 31 oxen.

(B) Blood-Urea (Using Ambard's Method)
Average, Normal Cattle, Uganda
(70 tests) 44·77 mllgm. per cent.
Minimum: 23·0 mllgm. per cent.
Maximum: 75·0 mllgm. per cent.

These tests comprised 21 cows, twelve calves, and 37 oxen.

It will be seen from the above that the blood-sugar value obtained corresponded closely with the results obtained by other workers up to 1927; but subsequent workers have found wide variations from these figures. FISH[5] and HAYDEN and FISH[6] and HAYDEN[7] found slightly lower values, ranging from 0·0412 per cent. to 0·0465 per cent.; while SCHLOTTHAUER[8] gives 0·0655 per cent.; AMADON[9] 0·0685 per cent.; MAGUIRE[10] 0·08 per cent. to 0·12 per cent.; HEWITT[11] 0·096 per cent.; and SJOLLEMA[12] 0·085 per cent.

Taking the average of all the values quoted above, however, the figure obtained in Uganda approximates with the result, *i.e.*, 0·0665 per cent.

With regard to blood-urea, no further records have been traced since HAYDEN,[4] quoted above, with the exception of SJOLLEMA[12], who gives 31·8 mllgm. per cent. to 43·2 mllgm. per cent.

The results obtained in trypanosome cases are summarised as follows:—

	Blood-Sugar. Per cent.	Blood-Urea. mgm. Per cent.
T. Vivax.		
Ox No. 95, inoculated 20.1.27	9.12.26—0·068	—
Given " Cyclosan," 10.3.27	14. 3.27—0·094	151
	18. 3.27—0·060	72
	21. 3.27 —	50
Before " Halarsol " ...	25. 3.27—0·060	62
After " ...	26. 3.27—0·060	40
T. Congolense.		
Bull No. 101, after infection	11. 3.27 —	49
	17. 3.27—0·042	65
	18. 3.27—0·060	54
Bull No. 100, inoculated 8.4.27	9. 3.27 —	46
	16. 3.27—0·085	43
	25. 4.27—0·081	54
Bullock No. 2, Jinja ...	16. 8.29 —	30
" " 3 " ...	" —	31
" " 4 " ...	" —	20
Bull, No. 1, Entebbe ...	10. 9.29 —	70
" " 3 " ...	" —	73
Bull, No. 1, Singo ...	24.10.29 —	32·5
" " 2 " ...	" —	27
" " 4 " ...	" —	25
" " 5 " ...	" —	24
" " 6 " ...	1.11.29 —	27
" " 7 " ...	" —	26
" " 8 " ...	" —	30
" " 9 " ...	" —	27
" " 10 " ...	" —	24
" " 11 " ...	" —	17
" " 12 " ...	" —	70
T. Rhodesiense.		
Calf No. 13	16. 5.27—0·087	75
" " 19	" 0·075	75
" " 59	18. 5.27—0·058	—
T. Gambiense.		
Sheep, No. 316	18. 5.27—0·048	—

Examining the above figures, it will be seen that, in regard to blood-sugar, only one case of hyperglycæmia was observed (Ox 95) and then only after injection with " Cyclosan." No cases of hypoglycæmia are evident. It is not possible, from the small number of estimations made, to compare these results with those obtained by more recent workers. It is hoped to continue these investigations in the near future, however, when it may be possible to verify the premortal hypoglycæmia found by SCHERN, KURT, ROSSILEMA[13] and SCHERN[14]; and confirmed by BRUYNOGHE, DUBOIS and BOURKAERT[15], ZOTTA and RADACOVICI,[16] and TUBANGUI and YUTUC[17]. These latter authors agree with REGENDANZ[18] in that a progressive hypoglycæmia is not evident in trypanosomiasis. The phenomenon of premortal hypoglycæmia in the development of trypanosomiasis is particularly

well shown by TUBANGUI and YUTUC (*loc. cit.*) in their blood-sugar curves, where the fall in blood-sugar always corresponds with the rise in the number of trypanosomes in the peripheral circulation. This phenomenon, however, appears to occur in several other diseases, and it has not yet been proved that it is the ultimate cause of death, as held by SCHERN (*loc. cit.*).

With regard to blood-urea, only one very high value was observed (Ox 95), and here again the increase followed injection of "Cyclosan," and there was no coma. Some low figures are evident in the *T. congolense* cases, but without more complete records during the whole period of infection it is not possible to draw any conclusions.

Acknowledgment must be made to the courtesy of Dr. H. L. Duke, O.B.E., for his assistance in allowing tests to be carried out on the *T. rhodesiense* and *T. gambiense* cases; and to Mr. H. G. Stewart, M.R.C.V.S., for his co-operation in collecting samples from *T. congolense* cases.

Bibliography

[1]Hayden, C. E., and Sholl, L. B. The Blood and Urine of the Cow in Milk Fever. Rpt. N.Y. State Vet. Coll., 1923-24, pp. 91-101, and (*ibid.*) p. 102.

[2]Widmark, E., and Carlens, O. Parturient Paresis: A Hypoglycæmic Coma. *N. Amer. Vet.*, vi (1925), p. 28; translated from *Biochem. Zeit.*, clvi (1925), p. 454.

Auger, L. Recherches sur la Pathogéniè de la Fievre Vitulaire. *Rev. Gén. de Méd. Vét.*, xxv (1926), p. 353.

[3]Moussu, G., and Moussu, R. Glycæmie Normale chez les bovides, Glycæmie et Maladie Vitulaire chez les vaches laitieres. *Compt. Rend. Acad. Sci.*, clxxxiii (1926), 7, pp. 431-3.

[4]Hayden, C. E. The Blood and Urine of the Cow in Milk Fever, (Second Report.) Rept. N.Y. State Vet. Coll., 1924-5, pp. 200-205.

[5]Fish, P. A. The Physiology of Milk Fever. *Jour. Am. Vet. Med. Assoc.*, Vol. lxxiii (1928), pp. 10-38.

[6]Hayden, C. E., and Fish, P. A. The Normal Blood of some Domesticated Animals. *Corn. Vet.*, xvii (1928), pp. 197-203.

[7]Hayden, C. E. Sugar, Guanidine and Cholesterol in the Blood of the Cow in Milk Fever. *Corn. Vet.*, xix (1929), p. 285.

[8]Schlotthauer, C. F. Theories on the Etiology of Milk Fever. Experimental Investigations. *Corn. Vet.*, xvii (1928), p. 217.

[9]Amadon, R. S. Experimental Study of Milk Fever. *Univ. Pa. Vet. Ext. Quar. Bull.*, 18 (1928), p. 16.

[10]Maguire (quoted by Hewitt; *see* [11]).

[11]Hewitt, E. A. The Blood-Sugar Level of the Bovine. *Jour. Am. Vet. Med. Assoc.*, Vol. 77 (1930), p. 362.

[12]Sjollema, B. The Importance of Inorganic Salts in the Animal Body—Biochemistry of Milk Fever in Cows. *Biochem. Zeit.*, 200 (1928), pp. 300-308.

[13]Schern, Kurt, and Rossi-Lema. Sugar content of the Blood of Animals Experimentally Infected with Mal de Caderas. *Zentralbl. f. Bakt.*, 1 (1928), Orig. Vol. 108, pp. 394-7; *Jahresbericht Vet. Med.*, Vol. 48 (1928), p. 1012; *Berl. Tierarztl. Wochenschr.*, Vol. 44 (1928), pp. 368-9.

[14]Schern, K. *Zbl. fr. Bakt.*, Orig. Vol. 119 (1931), p. 297. Sugar-deficiency Intoxication in Trypanosomiasis.

[15]Bruyhoghe, R., Dubois, A., and Bourkaert. Le sucre du sang au cours des trypanosomiases expérimentales. *Bull. Acad. Roy. Med. Belgique*, 7 (1927) pp. 142-157.

[16]Zotta, G., and Radacovici, E. Contribution a l'étude du metabolism de glucose sanguin dans la trypanosomiase expérimentale; and Sur la variations de la glycémie dans la trypanosomiase. Reviewed by Yorke in *Trop. Dis. Bull.*, 27 (1930), pp. 242-3.

[17]Tubangui, M. A., and Yutuc, L. M. The Resistance and the Blood-Sugar of Animals Infected with *Trypanosoma evansi*. *Philippine Jour. Sci.*, Vol. 45 (1931), pp. 93-107.

[18]Regendanz, P. Zur glykopriven intoxikation bei der trypanosomiasis usw. *Zbl. fr. Bakt.*, Orig. Vol. 119 (1931), p. 297.

ON THE APPARENT NON-PATHOGENICITY OF THE TREMATODES *EURYTREMA PANCREATICUM* (JANSON 1889) AND *PLATYNOSOMUM CONCINNUM* (BRAUN 1901)

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1. *EURYTREMA PANCREATICUM*

During February of this year Mr. M. N. K. Kurup, veterinary inspector, and Mr. K. Raman Nair, veterinary assistant, at my suggestion kept records of the number of flukes found in the pancreas of 34 consecutive bovines slaughtered for human food, with the following results:—

Date of slaughter in February, 1933.	Sex.	Condition.	No. of flukes in pancreas.
2	—	V.G.	1,202
3	—	P.	781
4	—	G.	258
4	—	P.	1,341
5	cow	P.	2,008
6	bull	G.	Nil
7	cow	G.	519
7	cow	G.	1,876
8	cow	G.	408
8	—	P.	1,327
9	cow	V.G.	441
10	bull	V.G.	887
11	cow	G.	378
12	—	V.G.	219
13	cow	P.	128
13	bull	G.	2,225
14	bull	V.G.	533
14	cow	G.	1,953
15	cow	P.	169
15	bull	V.G.	172
16	cow	G.	1,849
17	cow	G.	1,814
18	cow	V.G.	99
18	cow	V.G.	331
19	cow	V.G.	323
20	bullock	G.	97

V.G. = very good; G = good; P = poor.

Date of slaughter in February, 1933.	Sex.	Condition.	No. of flukes in pancreas.
21	bull calf	V.G.	Nil
22	bullock	V.G.	1,559
23	bull	V.G.	642
24	cow	V.G.	1,573
25	cart bullock	P.	273
26	bull calf	V.G.	1,183
27	bullock	V.G.	1,566
28	cart bullock	G.	102
			28,236
	Average	infection	830·4

V.G. = Very good ; G. = Good ; P. = Poor.

The sex is not given in the case of six animals and in no instance was the age ascertained. Thus it will be seen that 34 animals, some of which were very good, some good, and others poor in condition, harboured 28,236 flukes in their pancreas, with an average of 830·4.

No. of animals.	Condition.	Total No. of flukes.	Range. From	To	Average.
15	V.G.	10,730	0	1,573	715·3
12	G.	11,479	0	2,225	956·5
7	P.	6,027	128	2,008	861·0

This analysis appears to indicate in a rough manner that *Eurytrema pancreaticum* does not produce very marked alteration in the condition of the animal. It will be noticed that the animals which were reported to be in very good condition harboured an average of only 145·7 flukes less than those which were said to be poor in condition; and further, that the animals reported to be in good condition had an average of 95·5 flukes more than had those in a poor state.

2. *PLATYNOSOMUM CONCINNUM.*

This fluke usually occurs in small numbers in the cat. In the case in question an apparently healthy female, somewhat on the thin side, and which had been weaning a kitten, was subjected to *post-mortem* examination. My usual experience has been that this fluke, when in small numbers, produces hardly any appreciable change, but in this case there was marked thickening and enlargement of the bile ducts in the liver associated with the presence of thousands of these flukes. The gall bladder was heavily infested, as also was the small intestine. In the latter organ one usually finds from two to five flukes, but in this instance there were about 200 in that site; there were also a few in the stomach, and although they may have been regurgitated from the small intestine, this is the first time I have encountered this parasite in that organ.

It would appear, therefore, that a heavy infestation of this species can bring about thickening and enlargement of the bile ducts, although this is rare and heavy infestations such as the present one do not seem to be common.

The Veterinary Record

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WATER-TIGHT COMPARTMENTS

In a letter published recently in *The Times* (October 2nd) Dr. J. A. Arkwright, Chairman of the Committee of the Agricultural Research Council on Brucella Infections in Animals and Man, makes the following statement: "The long delay in recognising the close resemblance between the two bacteria [*Brucella melitensis* and *Br. abortus*] was no doubt in great part due to the water-tight compartments in which human and veterinary medicine were then segregated in this country. Happily, a new era of co-operation between the two fields of comparative medicine is now in progress . . . ." And he goes on to mention the composition of his committee, and the directions in which this happy band hope to spend their energies in the new era opened up by their industry, wherein they will announce to the world, among other things, the best means of diagnosing infection with *Br. abortus* in cattle. Dr. Arkwright's letter is inserted as a commentary on a letter published by Lord Winterton in *The Times* recounting his somewhat unpleasant experience in contracting infection from drinking milk contaminated with the abortion organism.

The reasons for the delay in recognising the resemblance between the bacteria which are the causes of the human and bovine diseases can naturally be appraised properly only by bacteriologists who have had experience in the problem at issue. We cannot, however, allow that the delay was attributable in great part, or even in any part, to workers in veterinary medicine in this country having segregated themselves in a water-tight compartment, whatever significance that well-worn *cliché* may have here. If it means that veterinary surgeons in this country have in the past minded their own affairs, we feel still, even with the advent of the new era, that our progenitors acted wisely, and that their action did not retard by a day the recognition of the above-mentioned resemblance. The eventual discovery, largely fortuitous, in the laboratory that the bacteria

bore such close resemblance brought to light, it must now be generally admitted, a very remarkable and rather surprising phenomenon, in that the causal agent of a most extensive disease in temperate countries of one subject, namely, the cow, which suffered from abortion as a result, was allied to that of a disease, confined, as it was thought then, to certain sub-tropical regions, of another subject, namely, man, who suffered from a peculiar prolonged fever—Malta fever—as a result. At this late hour, we can easily point from the reports of the early workers on Malta fever in goats to how suspicion might have been aroused from the clinical findings in these animals, especially after the discovery was made by one of the workers on the Malta Fever Commission, as Dr. Arkwright rightly points out, that some London milks reacted to the test which had been applied to detect infection in the milk of goats in endemic areas.

If there was any tardiness in the discovery, having in mind its wide implications when it was made, we cannot but suspect that there were at least two important contributory causes, namely, (1)) that on the medical side the bacteriologists had been content to leave their organism with the appellation, a micrococcus, with which it had been originally misnamed, and (2) that among the many fevers which medical men had relegated to the category of pyrexias of unknown origin, they had not unravelled one, in this country at any rate, which suggested a resemblance with the well-described Malta fever and which would have enticed them to pursue their enquiries further.

We need not labour these points, as their significance will be readily appreciated by interested readers, except that we contend that, so far as they may constitute a reproach, it is not one that can be legitimately levelled at the veterinary profession. Rather do we think that water-tight compartments are structures of the imagination which do not enclose our profession or tend greatly to sub-divide its activities, ministering as it does to many living subjects, the ills of which are readily amenable to the experimental method of investigation; but this profession, as always, is ever ready to collaborate on matters affecting the public weal.

Mr. E. A. Williams, cattle dealer, Culmstock, Devon, writes to *The Meat Trades Journal*: "I read in the *Journal* that a beast killed at Stockton had kidneys that weighed 10 st. 4 lb., but I can beat that. I have killed a beast with kidneys weighing 240 lb."

Clinical and Case Reports

MESENTERIC TEAR WITH STRANGULATION IN A FILLY*

E. BRAYLEY REYNOLDS, O.B.E., M.R.C.V.S.
Newmarket

A yearling thoroughbred filly had an attack of colic, lasting a few hours only, the pain being very acute and almost continuous while it lasted. An ordinary colic drink was given, she got out of pain, and was apparently none the worse. The filly had recently been broken, having been tacked about a fortnight previously. It had particularly resented the tacking, plunging and kicking very vigorously—more so than most animals under similar circumstances. Ten days later I was asked to see this filly, as it was lame. I was told that the day before it had been too lame to move about the box and that it would not be able to trot. It was walked round the yard twice and a trot was got out of it. I would not like to say that the filly seemed more lame in front than behind, but it did appear to be stiff in its back when turned. I did not attempt a definite diagnosis. Four days later I was called to see the filly as it had been found dead in its box.

By a number of small contusions on various prominent parts of the body and by the disturbed state of the litter, it was very apparent that the filly had had another attack of severe pain. There appeared to be definite evidence, however, that death had occurred very suddenly. The body was in a somewhat unnatural position, as though the animal had fallen dead; it was not flat on its side with the legs extended, but rather crouched together; nor had any movement of the limbs occurred after the animal had fallen, as shown by the quite undisturbed state of the litter in immediate contact with the limbs. The visible mucous membranes were very blanched.

Post-mortem Examination.—A *post-mortem* examination was made a few hours later. On arriving at the knacker's yard I was greeted with the remark: "This carcass might have been sent across the water." The skin had been removed and the carcass was very pale and clean-looking; there was no trace of any blood—it had the appearance of that of a stuck animal.

On opening up the abdomen the intestines presented a very noticeable appearance. The whole of the small intestine was a dark chocolate colour, and this was intensified by the very pale

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* Case presented to the Eastern Counties Division, N.V.M.A., on the occasion of the Summer meeting held at Great Yarmouth, on July 21st, 1933.

appearance of the large intestine and the subperitoneal fat of the abdominal wall. The whole of the intestines were removed intact, as far as possible. A closer examination revealed that there was a small slit in the mesentery under the crook of the cæcum, about six inches from where the ileum enters the large intestine. The whole of the small intestine, with the exception of the first ten feet of it, had passed through this tear, which was about three inches long and did not appear to be of quite recent occurrence. The edges of the tear showed no sign of hæmorrhage but were quite healed over and smooth and glistening. There was no sign whatever of any adhesion or threatened adhesion of the bowel, but this was freely movable through the opening in the mesentery. The folding of the mesentery of the invaginated portion of the bowel close to the opening through which it had passed had caused such compression on the thin-walled veins as to prevent any outflow of blood, but not sufficient to stay the forcing in of blood along the thicker-walled arteries. The wall of the strangulated portion of the small bowel was from about three-eighths to half-an-inch thick, and its lumen was filled with a sanguinolent fluid.

In view of the bowls competition awaiting decision I do not propose to say anything further, but I hope I have said sufficient to promote a short discussion. The main points that suggest themselves to my mind for this purpose I will submit in the form of questions: (1) Can tearing of the mesentery occur as the result of violent exertion? (2) Can a partial strangulation of the small bowel become rectified of its own accord? (3) Can strangulation of a large portion of the alimentary tract cause the withdrawal from the circulation of a sufficiently large amount of the blood to cause death by collapse as apart from shock resulting from the pain caused by the strangulation?

Discussion

Mr. T. G. Heatley said it was a very interesting case. There might have been quite enough blood pumped into the small intestine to cause a deficiency of blood in the general circulation to account for death from heart failure. It was very difficult to say whether rupture of the mesentery could take place through violence. Did it occur at the time when the filly was first bitted and she was fractious? With regard to Mr. Reynolds' last question, when the filly got the first attack of colic there was temporary obstruction of the flow of the fæces. Then the next attack came on, and the bowel became congested to such an extent that the veins were congested. Was not that how the blood came in?

Mr. W. Shipley said the real point of interest was twist, so-called, more particularly referring to the small intestine. He once asked his father, "Why let a horse roll when it has colic, because it is bound to get a twist?" His father's reply was that a horse's intestines were not thrown in like muck onto a wheel-barrow. What was twist of the small intestine? In his opinion, the cause of death was syncope produced by toxæmia. It was likely to happen with incomplete circulation. There had been an absorption of toxins through strangulation when the animal was ill a second time. The tear originated in an attack of colic. The colon was not involved, and there was irregular peristaltic action of the small intestine; hence the pain. During irregular peristaltic action the tear took place. By anodyne treatment the pain was relieved, but the tear was still there. There were no ill-effects during the time that this intestine gradually and quietly worked through, unfil the whole of it was through, when it became strangulated. That was twist, possibly from some violent exertion, such as the animal throwing itself down, or suddenly slipping. The tear happened, and was the origin of twist. Rupture of the mesentery must take place before twist.

Mr. Heatley said he did not agree that they could not get twist without rupture of the mesentery.

Mr. Shipley replied that his view was that twist of the intestine was strangulation, and by strangulation he did not mean that the bowel was tied in a knot.

Mr. P. R. Turner said the rupture might have occurred previously, and the venous congestion of the bowel became progressive. If that was so, one would have thought there would have been some evidence of gangrene in the involved portion of the small bowel, if it had been going on for ten days.

Mr. W. Turtill said he should think the filly got hurt when the men were breaking it, and the bowel gradually worked through.

The Reply.

Mr. Reynolds, in reply, said that the particular lesions, as revealed at the *post-mortem* examination, were unique in his experience, and it was their peculiar character that had excited his curiosity to arrive at a satisfactory solution, if possible, of their cause and proper sequence.

His queries might be taken as suggesting his final conclusions. Whether tearing of the mesentery could result from violent exertion was, perhaps, just mere guesswork. The abdominal cavity being, in fact, only a potential cavity, and the whole of its contents being held in close apposition, it was difficult to understand how tearing of the mesentery could occur. If tearing were possible, it seemed to him it was more likely to occur in the upper part where it was firmly adherent and its movement limited than towards its expanded, more freely movable margin where connected to the small bowel. He would not like to suggest that tearing, as the result of violence, would be more likely in the particular position it had occurred in this case, but he did wonder whether it might have been caused by the sudden movement of the first part of the colon away from the more or less fixed crook of the cæcum. If the first attack of pain had been due to a short portion of the bowel having passed through the tear and become temporarily strangulated, he thought this must have righted itself, as he could not think that the bowel had sufficiently adapted itself to this abnormal position for 20 days, and that the more aggravated strangulation had then been set up by a much longer portion of the bowel passing through the opening. The manner in which the small bowel had passed through this opening might be likened to that in which descent of the bowel occurred following the castration of a colt in which a lesion was not already present. A small

knuckle of bowel passed through the opening and the remainder followed by reason of its own peristaltic action.

He did not agree with Mr. Shipley that death was due to toxæmia. In the first place, he did not think sufficient time had elapsed between the onset of the symptoms in the last attack and death for this to have been the cause. The actual duration of the attack was not known, but that its onset must have been late in the evening was indicated by the fact that the filly had eaten the whole of its hay as well as its corn; and that death had occurred early was shown by the condition of the carcass when found at 6 o'clock in the morning. Nor were there any other indications of toxæmia; there was no sign of peritonitis. What peritoneal fluid there was present was normal in amount and colour; there was no adhesion of the bowel when it passed through the tear in the mesentery, the two ends of the bowel were freely movable through the opening.

He also found himself in disagreement with Mr. Shipley in regard to his remark that there must be rupture of the mesentery in "twist." Tearing of the mesentery was not always present in these cases, and he was satisfied that one was justified, in attempting to describe the condition of the bowel in some of them, in using the expression it was "tied in a knot." He had purposely avoided using the term "twist" in describing the case, as he had thought it totally inapplicable. In his view, this case was typically one of simple strangulation in contrast to the more usually occurring cases known as twist. He considered the cause of death was the withdrawal of the blood from the circulation by the imprisonment of it in the great length of the small intestine. He was forced to this conclusion for two reasons. The first and chief one, and one that was an unquestionable fact, was that the whole of the body with the exception of the small intestine was practically devoid of blood. The second reason was that the holding capacity of what was practically the whole length of the small intestine did not render this withdrawal as a cause of death an impossibility. He was quite prepared to admit that, had the withdrawal of blood not been so complete, the animal would eventually have died from shock and toxæmia.

Mr. Heatley asked whether this defect in the mesentery was congenital.

Mr. Reynolds said he could hardly think so, although the case, undoubtedly, was one of very unusual character.

Mr. Shipley said his statement was that the filly died from syncope or heart failure, due to toxæmia, but not general toxæmia. How much blood could be taken from a yearling and leave it alive?

Mr. Reynolds: "A bucketful, but it might depend on the size of the bucket."

Mr. Shipley asked whether there was a bucketful of blood in this portion of the intestine.

Mr. Reynolds replied that it was more likely there were two bucketsful. The wall throughout practically the whole length of the invaginated portion of the small bowel—roughly about 60-ft.—was, on *post-mortem* examination, found to be about half-an-inch thick and the distended lumen of the bowel was about 2-in. in diameter; one could, therefore, by a simple mathematical calculation, roughly estimate the volume of blood that could be imprisoned. It must be remembered, too, that there was no appreciable amount of blood in any other part of the body. He thought the filly had fallen unconscious from loss of blood from the circulation and that the cause of death was syncope, aggravated possibly by shock. Shock, in his opinion, played a great part in causing death in cases of bowel trouble in the horse, in addition to that exerted by toxæmia and other factors.

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FOUR CASES OF LIVER DISEASE

W. F. ASTON, M.R.C.V.S.
Shrewsbury

Case I: *Subject.*—A black Shire mare, twelve years old. She was working in the town and there was no knowledge of illness for five years. When turned out to grass for the week-end she galloped round and fell heavily; she was seen to move away stiffly and start to graze. Three days later she was suffering from diarrhœa; there was little appetite, her temperature was 103·6°, and her mucous membranes were injected—not exactly icteric until the next day, and this disappeared two days later. She moved very stiffly. Back slightly arched. Her temperature dropped to 102·0° during the fifth day, but the diarrhœa increased. On the sixth day she developed abdominal pain and enteritis and died that night.

Post-mortem Examination.—This showed the liver to be enlarged, a fibrous surface around a calculus which filled a large sac of the gall duct at the portal fissure. The inner and outer surfaces of this sac were undergoing inflammation. There were areas of peritonitis adjoining and extending to the curvature of the large bowel, where it appeared more active. A patchy and recent inflammation of the colon was the only other lesion.

The gall-stone was the size of a small cocoanut, very distinctly layered and fractured in two large and three small pieces.

Case II: *Subject.*—A two-year-old thoroughbred filly. She had been in poor condition as a yearling, and had received extra food during the winter. In July, 1933, I was consulted because of intermittent diarrhœa and emaciation, and a slight eversion of the rectum, which partly responded to treatment. Fæcal examination for strongylus eggs was positive. The filly was treated for these, but her condition grew no better, and she was destroyed three weeks later. There were no roundworms in the alimentary tract.

Post-mortem.—This examination showed the liver to be very enlarged, perfectly marbled, very fibrous and firm to touch and cut; its weight was 48 pounds. There was an aneurysm in the mesenteric artery. There were no roundworms in the alimentary tract.

Case III: *Subject.*—An Ayrshire cow, seven years old, calved four months. I was consulted because the cow had gone off her food. The action of the bowels was normal, her temperature was 106·0°, her pulse 60. She was treated

with a simple febrifuge, and the usual stomachic powder prescribed. Two days later, although eating a little, the condition was still unsatisfactory, and on auscultation there appeared to be a commencing pleurisy at the bottom of the right lung. Twenty-four hours later the pleurisy was more marked. The temperature was still 106·0°. There was a groan on movement, and resting of the head on manger and ground. Respirations were not accelerated, but shallow and painful. The bowels were acting normally. The cow died next morning.

Post-mortem.—Revealed multiple carcinoma of the liver with secondary pleurisy affecting the right lung and diaphragm. The growths were from the size of a hazel nut to that of a walnut, white in the centre, becoming nearer the colour of liver substance at the periphery. The whole tissue of the liver was embedded with these growths, which in some places had coalesced.

Case IV: *Subject.*—A Hereford bullock two-and-a-half years old, one of 20 purchased in the spring and out at grass all summer. After four months this animal was noticed not to be making the same progress as the others; within a week his appetite was failing and he was got in.

Clinical examination showed slight diarrhœa, with straining. Temperature 102·0°; he was bright, would eat a few mouthsful and then desist. The lungs and heart were normal, the pulse rather slow. A purgative was administered, followed by the usual treatment for diarrhœa. For three days, in the owner's opinion, a satisfactory response occurred. On further examination he was seen to be losing flesh, and the straining became more marked. After six days he was destroyed.

Post-mortem Examination.—This showed the liver to be enormous—nearly 60 pounds, very firm and leather-like to cut. The bile ducts and blood vessels were well defined by a layer of fibrous tissue. The substance of the liver appeared normal in colour and was not interspersed with fibrous tissue. There was about one pint of bile in the gall bladder. There was no trace of fluke or even of a previous infection. All the other organs were healthy.

The persistent straining in this case was the symptom which alone enabled a definitely unfavourable prognosis to be given.

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LARYNGEAL CYST IN A HORSE

A. R. SMYTHE, F.R.C.V.S., D.V.S.M.
Falmouth.

An aged cab horse was sent in one day for examination. The driver stated that he was exercising the horse at a trot in the early morning, when suddenly dyspnœa developed, and the horse fell to the ground.

After a few minutes he appeared to become easier and was got up and led back to the stable.

The horse had been used for posting work regularly, and had worked on the previous day.

The usual clinical examination revealed nothing to account for the incident but, on being exercised at a fast trot, the animal again fell, and appeared to be suffocating.

The horse was therefore cast and chloroformed and an examination made of his throat. A large pedunculated growth was encountered, on the upper surface of the epiglottis, which was removed with the aid of a wire écraseur.

The growth proved to be of a cystic nature of about the size of a hen's egg. Being attached to the epiglottis by the flexile mucous membrane, it became drawn into the laryngeal opening, when the respirations became more forcible.

The horse made an uninterrupted recovery.

Reports

NEW SOUTH WALES DEPARTMENT OF AGRICULTURE LIVESTOCK DISEASES REPORT (No. 8)

RECORDING CONTROL WORK DURING THE YEAR ENDED JUNE 30th, 1932

It is to be regretted that, for financial reasons, the issue of a report of equal magnitude to those previously published has not been possible, but nevertheless Mr. Max Henry, Chief Veterinary Surgeon, has presented an abridged report of considerable interest.

He first discusses the effects of a recent reorganisation of the Department, which has proved satisfactory, and then deals with the work performed at the Glenfield Veterinary Research Station, which has now reached such proportions that it has become beyond the capacity of the staff. It is satisfactory to note that the Council of Advice of the Pastures Protection Boards has decided to continue the subsidy for research at the station for a further period of five years, while additional help is anticipated from a scheme for research into the diseases of dairy cattle from the United Pure-bred Dairy Cattle Breeders' Association. A table is given showing the number of stock crossing the borders of New South Wales to and from neighbouring States and the number of animals treated by dipping and spraying with arsenical solutions for the destruction of ticks.

During the year there were 86 outbreaks of bovine pleuro-pneumonia, involving 9,566 cattle, of which 312 died or were destroyed. Quarantine, repeated inspections, and protective inoculation were generally successful in eradicating the disease within twelve months. The number of herds inoculated as a preventive measure during the year was 106, comprising 7,807 animals. The number of outbreaks of anthrax was 29, involving 79,251 sheep and 161

cattle. The total number of animals vaccinated during the year was 197,874 sheep, 11,431 cattle and 26 horses. One District Veterinary Officer considers that he is able to associate the incidence of the disease with grass seed infestation. Sixty-eight outbreaks of blackleg were recorded and 1,794 animals were destroyed for tuberculosis. Under a tubercle-free herd scheme 4,554 head of cattle were tested, of which nine reacted. The distribution of piroplasmosis is discussed and the seasonal incidence of black disease is explained. It appears that when a good autumn follows a "green" summer the losses are heavier than after a dry summer, due to the fact that fat and good-conditioned sheep are more liable to infection.

During the year it was decided to provide for the publication of a list of abortion-free herds in the *Agricultural Gazette* on lines similar to the action taken regarding tubercle-free herds. A long list of poultry diseases and other miscellaneous diseases such as mycotic dermatitis, bacterial degeneration of wool, tetanus, botulism, infections with *Bacillus necrophorus*, infectious nasal catarrh in sheep, malignant œdema, suppurative otitis in pigs, symbiotic mange in horses, ophthalmia in cattle and sheep, and abortion in sheep, terminates this interesting report. It is therefore unfortunate that "financial considerations" have prevented the publication of further details of these conditions, which would have proved helpful and instructive not only to veterinarians but to stock owners themselves. Ll. E. W. B.

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FOOT-AND-MOUTH DISEASE IN THE BECHUANALAND PROTECTORATE

REPORT FOR THE MONTH ENDING JULY 31st, 1933

The Chief Veterinary Officer of the Bechuanaland Protectorate, Mr. W. H. Chase, has favoured us with a further report of the work performed by his Department during the month of July, 1933, in its endeavour to control the outbreak of foot-and-mouth disease in that territory.

During the month the entire inoculating staff was transferred to the Bamangwato Reserve, an area to which public attention has recently been drawn by the misdemeanour of Tshekedi, one of the native chiefs of the district. The work in this Reserve has been speeded up, and all inoculations in the Protectorate should be completed by the end of August. At the end of June last 582,007 head of cattle had been inoculated by the intra-nasal blood-virus method introduced by Bevan in Southern Rhodesia in 1931, and the total inoculated at the time the present report was compiled was 718,000 head.

In order to ensure a supply of virus as and when required, lymph virus has been maintained at Mokoro during the month. Such a procedure, if adopted earlier, might have obviated some of the difficulties experienced in obtaining suitable blood-virus at the commencement of the inoculation campaign.

It is interesting to note that "cattle which were injected with lymph into the sub-epithelium of the feet between the claws showed extensive foot reactions and vesicles in the mouth four days afterwards." Also that "a herd which had become naturally infected at the commencement of the outbreak, and which was reported to have had the disease in severe form, was subjected to the intradermic injection of the tongue. 38·46 per cent. of these animals gave a positive reaction, showing that natural infection was approximately 60 per cent." A very interesting observation, having a bearing upon the relative value of inoculation as against natural contact infection.

G.V.O. Walker at Mokoro has carried out experiments in connection with (1) the transmission of foot-and-mouth disease in young calves, (2) the infectivity of blood of calves reacting to intradermal tongue inoculation, (3) the reactions occurring in calves, and (4) the immunity of recovered calves.

Ll. E. W. B.

N.V.M.A. Divisional Report

EASTERN COUNTIES V.M.A.

SUMMER MEETING AT GREAT YARMOUTH*

The Summer Meeting of the Eastern Counties V.M.A. was held at the Royal Masonic Assembly Rooms, Great Yarmouth, on July 21st. The President, Captain W. Waters, was in the chair.

Before the meeting about 30 members with their ladies partook of the luncheon.

After lunch the business meeting was held. In addition to the President, the following members signed the attendance register: Messrs. J. M. Buchanan, V. Boyle, J. W. Beaumont, A. G. Beynon, J. M. Currie, B. A. Claxton, W. H. Dennett, S. E. Hill, T. G. Heatley, T. Love, W. S. Jack, G. T. Matthews, N. F. Pollock, E. Brayley Reynolds, W. Shipley, H. P. Standley, senr., P. R. Turner, W. Turtill, W. H. Wortley and the Hon. Secretary.

The minutes of the Norwich meeting and of a special meeting held at Ipswich were read and signed.

Letters regretting inability to attend were received from Messrs. W. Blyth, H. Buckingham, J. Buckingham, Professor J. B. Buxton, Messrs. A. J. Beeson, W. J. Browning, A. F. Castle, F. H. Downie, F. L. Gooch, Sir Frederick Hobday,

* Received for publication, September 18th.

W. F. Howes, W. F. Loveless, H. Low, H. H. Leach, G. T. Murphy, C. C. Nesling, J. T. Thurston and P. Welch.

New Member.—Mr. A. G. Beynon was elected.

Next Meeting.—It was decided to hold the Autumn Meeting at Bury St. Edmunds on Friday, October 6th.

Presidential Address

The President then gave his inaugural address, in which he said:—

It is apparently the custom for a newly-elected President to give an address at the first available opportunity afterwards, and for the benefit of those who were not present at the special meeting at Ipswich in May, I should like again to express my thanks for the honour conferred on me.

It is not my intention to give you a learned and lengthy review of the veterinary profession; in fact, I only wish to discuss two matters, both of which are connected with the E.C.V.M.A.

Considering how scattered the members of our Association are, I have felt for a long while that we do not have sufficient meetings to keep our members interested. For instance, we have the annual meeting at Norwich in February—usually very badly attended and mainly given up to the election of officers for the coming year. Then we have our summer meeting of a social character, including a bowls tournament for the Shipley Cup, and finally a meeting in the autumn, usually held in Suffolk. I would like to suggest that in future we hold four meetings a year, as follows: in February, at Norwich; in May, at Ipswich; in July, at Yarmouth; and in October, at Bury, Cambridge or Newmarket, so as to enable all our members to be able to attend at least two meetings a year.

The other matter is connected with clinical articles in the *Veterinary Record*, which, in spite of constant appeals, do not seem to increase in number. There are several reasons for this, but I think the chief one is that the majority of us feel self-conscious at rushing into print, and I would like to suggest that every member of the E.C.V.M.A. be asked to guarantee one clinical record a year, which could be sent through our representative, Mr. E. B. Reynolds, or direct to the *Veterinary Record*. If we made the promise, as soon as we had an unusually interesting case we would write it up at once, so as to be ready with the contribution as soon as required.

If this suggestion seems practical, I am willing to contribute one "clinical" a year in the hope that you will also follow suit. As I have nothing further to add, perhaps you would like to discuss the matters I have suggested.

Mr. Shipley pointed out that the number of meetings was fixed under the rules, but notice could be given now to move an alteration of the rule at the next meeting. It would not be desirable to specify the towns in which the meetings must be held.

The President then gave notice that at the next meeting he would move such an alteration.

The Hon. Secretary informed Mr. Shipley that there were 66 members in the Association.

Mr. Shipley pleaded for exemption from supplying a clinical article on the ground that there are only 52 weeks in the year. (Laughter.)

The President: "I had hopes of seeing half-a-dozen articles in an issue instead of one." (Hear, hear.)

An Interesting Bowel Case.—In introducing this case (which is recorded, with ensuing discussion, in the Clinical Section of this issue of the *Record*), Mr. E. Brayley Reynolds said he was glad to hear the President make the suggestion that each member should supply a clinical article to the *Veterinary Record*. He was greatly surprised when he received the notice of the meeting to find he was the only individual put down for a paper, as from a letter he had received from the Hon. Secretary last June, he understood there were to be two others. They apparently had fallen out, and this did not look very promising for articles for the *Record*. Perhaps these gentlemen took the Secretary's reminder that that meeting was mainly of a social nature too literally, and did not wish to encroach upon that side of it. He would suggest that the two gentlemen who were not submitting their notes to this meeting should submit clinical articles for the *Record*, though he knew it was often difficult for a veterinary surgeon to find time to sit down to write up a case. He had put down a few notes of a case that had recently come under his notice, the discussion of the various points of which could unfortunately only be of purely academic interest, and would in no way add to their knowledge for the better carrying out of some of their daily work—he had looked for some contribution toward this latter from the others. The case, however, presented several points of interest and appeared to be worth recording.

Fowl Cholera.—Mr. Matthews called attention to the very serious outbreak of fowl cholera on the borders of Norfolk and Suffolk. The agricultural organiser told him that 95 per cent. of the work of his poultry instructor related to the diseases of poultry, and he was astounded that he could not get more help from the veterinary profession in this matter. He wished veterinary practitioners would help him, as he did not wish his poultry instructor to take up diseases at all. There was a big field for the veterinary profession if they cared to take it up. The Ministry of Agriculture looked to the profession to give advice and treatment to the poultry keeper.

This ended the business, and a bowls tournament followed for the Shipley Cup, which

is competed for annually. This resulted in a win for Mr. W. H. Dennett.

The subscription on behalf of the Victoria Benevolent Fund in connection with this tournament amounted to £3 12s. 6d.

At the conclusion of the tournament members and their ladies were kindly entertained to tea by the President and Mrs. Waters.

The meeting concluded with a hearty vote of thanks (amidst applause) to the President for his hospitality and the able manner in which he had conducted the meeting.

H. P. Standley, jun., *Hon. Secretary.*

Notes and News

The Editor will be glad to receive items of professional interest for inclusion in these columns.

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DIARY OF EVENTS

Oct. 23rd.—Meeting of the Editorial Committee, N.V.M.A., at 2, Verulam Buildings, Gray's Inn, W.C.1, at 4.30 p.m.

Oct. 25th.—Meeting of the Lancashire Division, N.V.M.A., at Liverpool.

Oct. 25th.—Meeting of the Scottish Metropolitan Division, N.V.M.A., at Edinburgh.

Oct. 25th.—Meeting of the Section of Comparative Medicine of the Royal Society of Medicine, 1, Wimpole Street, W.

Oct. 27th.—Meeting of the Yorkshire Division, N.V.M.A., at Leeds.

Oct. 28th.—Meeting of the N.V.M.A. Special Committee on Small Animal Euthanasia, at Liverpool.

Nov. 2nd.—Central Veterinary Society: Annual Dinner at the Mayfair Hotel, W.

Nov. 3rd.—R.A.V.C. Old Comrades' Association Annual Meeting and Dinner, Anderton's Hotel, Fleet Street, E.C.

Aug. 13th-18th, 1934.—Twelfth International Veterinary Congress, New York.

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WHOLE-TIME VETERINARY OFFICER FOR ESSEX

At a meeting of the Essex County Council on October 3rd, a recommendation (of a joint subcommittee of the Public Health Committee and the Diseases of Animals Committee of the Agricultural Committee) to appoint a whole-time chief veterinary officer for organising and superintending the duties of the Council's veterinary officers, and generally advising the Council and Committees on matters relating to the Council's veterinary service, was approved. They recommended a minimum commencing salary of £700 per annum, rising by annual increments of £50 to a maximum of £1,000.

The Public Health Committee approved the proposal, but accepted the suggestion of the General Purposes Committee that the salary should rise to a maximum of £900. The Agricultural Committee, however, recommended that the maximum should be £1,000.

The suggestion of the General Purposes Committee that the salary should be a maximum of £900 was accepted, on the understanding that if in a few years' time the officer proved thoroughly satisfactory, and the work developed, there should be no bar to the reconsideration of the question of the maximum.

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FOOT-AND-MOUTH DISEASE

During the past week confirmation has been made of outbreaks of foot-and-mouth disease at Barkway, near Baldock, Hertfordshire, and at Ely, in the Isle of Ely. In the former outbreak both cattle and swine were involved.

As a consequence of these outbreaks there is now an area of restricted movement 60 miles in length—30 miles north and south of Cambridge, and many miles in width. One effect of the Baldock outbreak was the destruction of Sir Humphrey de Trafford's famous herd of Red Polls.

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WILD LIFE IN AFRICA

It is officially announced that an International Conference to consider measures for the protection of the fauna and flora of Africa will meet in London on October 31st. The Conference will meet in the House of Lords and will be presided over by Lord Onslow, who will also be the chief delegate of His Majesty's Government in the United Kingdom.

The Government will also be represented at the Conference by Sir William Gowers, Senior Crown Agent for the Colonies, Sir Arnold Hodson, the Governor of Sierra Leone, and Mr. A. B. Acheson, of the Colonial Office. There will also be representatives of the Governments of South Africa and Southern Rhodesia.

Other Governments that will be represented are the Governments of Abyssinia, Belgium, Egypt, France, Italy, Portugal, Spain, and the Anglo-Egyptian Sudan. The Governments of India, the Netherlands, and the United States have nominated observers to attend the Conference.

The Secretary of the Conference will be Mr. Frances Hemming, Joint Secretary, Economic Advisory Council. The Assistant Secretary of the Conference will be Mr. D. H. F. Rickett, Assistant, Economic Advisory Council. The address of the secretariat will be 2, Whitehall Gardens, S.W.1.

The chief task of the Conference will be to draw up a revised international convention for the protection of the fauna and flora of Africa. It will be recalled that a resolution was passed at the International Congress for the Protection of Nature, which was held in Paris in the summer of 1931, urging that the question of the negotiation of a new international convention should be considered by the Powers concerned.

A draft Convention has been prepared by the British Government and circulated to the other Governments participating in the Conference to be used as a basis for discussion. This draft contains suggestions for an agreed declaration of principle on the subject of the establishment of national sanctuaries in which wild animals

and plants may be preserved with due regard to the interests of the native inhabitants of the various territories concerned. It contemplates the adoption of concerted measures of control designed to restrict the killing of some of the rarer species of animal in Africa, and to prohibit the unregulated traffic in trophies obtained from such animals. It also deals with various objectionable methods of hunting and other practices which have a destructive effect upon wild life, and is designed to secure co-operation between the various administrations concerned and the free exchange of information on all questions relating to the protection of the natural fauna and flora of Africa.

It is expected that the plenary sessions of the Conference will be held in public.

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A POSTER AT LIVERPOOL

A recent issue of *The Times* contains a vigorous protest by Major-General Sir John Moore, K.C.M.G., C.B., F.R.C.V.S., against a large poster displayed on the site of the new Mersey Tunnel entrance, Liverpool, in which, under the heading "Death Comes Again!" the statement is made that, whereas in 1914, 70,000 children died from infantile paralysis due to flies bred in horse dung, in 1930 only 3,000 children died—"63,000 lives saved by motor transport"!

Sir John points out that infantile paralysis (acute anterior poliomyelitis) spreads along the path of human intercourse only, infection resulting from those who have contracted the disease, or who are carriers of the causative micro-organism. It is communicated from one individual to another. No other factors in the spread of the malady are known, and there is no proof of the transference of the disease by fomites, vermin, flies, or other outside media. "It is therefore quite wrong to incriminate horses or horse dung as a causal agency," he adds. "Horses do not suffer from acute anterior poliomyelitis, and their excrement cannot be held to blame."

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"SOMETHING FOR NOTHING!"

In the current issue of the official organ of the R.S.P.C.A. (*The Animal World*), the Assistant Secretary, Captain Fergus MacCunn, in a useful article under the above heading, states that it is a very real pity that negotiations between the Society, either as represented by its Headquarters or its branches, and the veterinary profession, in regard to the establishment of dispensaries, or the decentralised scheme, should so often be hindered or complicated by the question of the abuse of the facilities offered, by people who can afford to pay professional fees.

"Undoubtedly, some people who can afford ordinary veterinary surgeons' fees do take advantage of the facilities provided by the Society for the benefit of poor people only, and one must realise the veterinary surgeon's point of view when he enters a vigorous protest against this contemptible practice," says the author.

"On the other hand the experience of the Society, through its dispensaries, up to date has been that the actual proportion of such people is very small compared with the genuinely poor owner, who, incidentally, is usually exceedingly generous as regards his donation to the dispensary collecting box.

"The essential difficulty in connection with this matter is to decide what is the correct procedure to adopt when a person who is obviously able to pay a veterinary surgeon's fee arrives at a dispensary intended for poor people only and produces a sick or injured animal for attention.

"The natural course would seem to be to explain politely but firmly that the dispensary exists solely for the animals of poor people, and having provided the particular visitor with the name of the nearest veterinary surgeon, politely show him out. In point of fact, this is the method which, on occasion, has been adopted in the past, but in the opinion of those who are closely connected with dispensary work nothing could be more inadvisable *from the point of view of the sick or injured animal itself*. It is surely logical to assume that a person so mean as to attempt to obtain free treatment, knowing that they have no moral right to it, is most decidedly not the type of person who, if refused such treatment, would take his animal to a veterinary surgeon and expend the necessary money. What is much more likely to occur is what, in point of fact, has been seen to occur on more than one occasion when treatment has been refused. The indignant owner, hurling abuse at the Society, has, on leaving the dispensary, taken the animal patient direct to a quack practitioner.

"The Society holds the view, and rightly so, that the animals of the poor have just as much right to the attention of a fully qualified veterinary surgeon as the animals of people in more fortunate financial circumstances. Further, that it is, to say the least, inadvisable in the highest degree, *in the interests of the animal*, that it should receive the ministration of any but a qualified man.

"Again, should the mean owner be refused attention and no unqualified man be available in the vicinity, it is also reasonable to deduce that the patient in that case will receive no attention at all. . . .

"There is, however, no possible reason at all why, if a person who is obviously able to pay the proper fees brings an animal to a free dispensary for treatment, that same person should not be informed, politely but firmly, that while the animal will receive exactly the same qualified treatment as any other creature brought to the dispensary, it is expected that he, or she, will make as generous a donation to the funds of the dispensary as possible. If this is done he must indeed be a thick-skinned owner who does not immediately respond, or who is not shamed into putting into the collecting-box, in the form of of a donation, something like the fee he would have been charged, had he gone, as he should have done, to a veterinary surgeon.

"No one would suggest that, in protesting, the veterinary surgeons are merely thinking of the fees they might lose through people visiting the free dispensaries instead of their own surgeries. The veterinary profession objects on a matter of principle, and very rightly, too. Their attitude is that, while they are willing to assist the Society for the benefit of animals of the poor in providing free qualified treatment, and in so doing to accept for their services a purely nominal fee, or no fee at all, yet they are decidedly not prepared to provide such assistance unless every care is taken to ensure that the facilities provided are not abused. Surely this is a very reasonable attitude to adopt, and one which should be readily appreciated by those, on behalf of the Society, who are responsible for the conduct of either a dispensary or a 'decentralised' scheme."

Correspondence

Letters to the Editor should reach the Office not later than by the first post on Tuesday morning for insertion in following Saturday's issue.

All correspondence must bear the name and address of the contributor for publication.

The Editor does not hold himself responsible for the opinions of his correspondents.

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SHEEP SLAUGHTER AND PSYCHOLOGY

To the Editor of the Veterinary Record

Sir,—Referring to your note under the head of "Sheep Slaughter Prosecution" in your issue of September 16th, Principal J. F. Craig's remarks seem to me to open up a line of thought which would be interesting to follow.

He expressed the opinion that the sheep would suffer no *cruelty* since they were unable to reason that what they saw happen to one would ultimately happen to another. In conjunction with this it should be noted that the charge inferred cruelty, but stated that 13 sheep were *terrified*.

The primary question seems to be not whether the sheep were cruelly treated, but whether or not they received the sensation applicable to fear, and whether this sensation, by being repeated, was sufficient to cause them distress to such an extent as to amount to their being submitted to cruelty.

There are two main schools of thought among psychologists, the one which follows Freud in placing Sex as the first of the primary instincts, and another which places Fear. There seems no reason why we should not consider, for the purpose of this case, the second, namely Fear. Further, there is some doubt as to whether fear is first manifest in a physiological or a mental manner. In either case the difference in time is so small that for all practical purposes they may be considered as being coincident.

On the first appreciation of a cause never before experienced—in this case the sight of slaughter, the smell of blood, the sound of a sheep in distress and the voices of men—a reaction takes place in the physical regulation of the subject. The heart begins to increase its activity, at the same time respiration becomes more frequent and other minor disturbances are evidenced. This is, in fact, an answer to the primary stimulus which had been transmitted to the subconscious by the three main senses, and is nothing more than an immediate "hotting up" of the engine preparatory to and for some immediate call which may be made on it. If no call is made, *i.e.*, if no action takes place warranting the expenditure of this increased reserve of energy, there takes place in the subject a sensation of oppression, of mental strangulation which is not very far removed from distress. Hence we may come to the conclusion that one sheep seeing another slaughtered suffers a certain amount of distress.

The question now resolves itself into whether this distress becomes more or less, according as the number of times the subject witnesses the event.

In the case of breaking in a horse to pass a steam roller, we cannot deny that fear is experienced in the first instance, but that after repeated presentations of the steam roller the horse begins to accept it as one of the ordinary phenomena of life comparable to the opening and closing of the stable door. Therefore, we gather that familiarity breeds, shall I say, "content." But we must not forget that the fear engendered in the horse is capable of being expressed in action: He is moved on, a use is found for the store of surplus energy. In the case of the sheep no action can, or does, take place.

To sum up, it seems that the sheep may suffer distress which is in the aggregate increased for a certain period of time but that after this period (and period is to mean number of occasions the slaughter of another is witnessed) a diminution in the intensity of the distress takes place.

The opinion of Principal Craig, that owing to the sheep's inability to reason it cannot appreciate a sensation of fear sufficient to amount to the infliction of cruelty, does not enter, since the sensation of fear is not primarily a matter of reason, but of sub-conscious reaction to some stimulus which engenders a state of physical discomfort, at the same time as, if not before, the same stimulus warns the conscious to "stand by" preparatory for immediate action.

Cruelty is a word difficult to define, but fear is not. In this case, the R.S.P.C.A. were right in prosecuting, in that distress was caused to the sheep through fear. Principal Craig may have been right in that he expressed the opinion that there was no cruelty, though his reason seems inadequate, and the judge would have done better had he made a deeper investigation into the psychological aspect before he dismissed the summons.

J. N. H. Bacon.

Royal Veterinary College,
Camden Town, N.W.1.

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A COMMITTEE OF THE ECONOMIC ADVISORY COUNCIL

To the Editor of the Veterinary Record

Sir,—I notice at page 1010 of the *Veterinary Record* for October 14th, under "Glasgow Milk Supply," that the Prime Minister has appointed a committee, as above, to report on the value and practicability of methods for reducing the incidence of bovine tuberculosis and improving the milk supply. Are any claims being put forward by our executive officers for adequate veterinary representation on this committee, or is it to consist largely of men who have no great knowledge of cows and cow-houses and whose fetish is solely pasteurised milk?

If a committee was formed to report on the value and practicability of methods for reducing the incidence of puerperal fever in women and improving the supply of healthy matrons and live youngsters, I do not think many veterinary surgeons would be invited to act on it—medical men would rightly be asked to serve and give of their knowledge and experience, and little else would be taken into consideration in forming repressive and therapeutic measures.

Equally, then, an adequate number, or a greatly preponderating number, of veterinary surgeons should serve on this committee. I wonder if it will be so, or whether a purely veterinary matter will be settled by theorists and romanticists and the public gulled once again? I should like to see the publication in the *Record* of the names of the persons appointed on this committee.

Yours truly,

G. Mayall.

Bolton.